# Cardiac Autonomic Assessment and Diastolic Function in Individuals with Spinal Cord Injury

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**Abstract**

The primary purpose of this thesis was to examine the validity and reproducibility of the QT-variability index (QTVI) as a measure of cardiac autonomic function in individuals with spinal cord injury (SCI). The secondary purpose was to investigate the influence of cardiac autonomic activity and blood volume on diastolic function in individuals with SCI. Results from **Study 1** showed that the QTVI increased when participants performed a cardiovascular (CV) stress maneuver, and subsequently returned to baseline after blocking sympathetic outflow via metoprolol. Moreover, while participants were resting, the QTVI increased after blocking parasympathetic activity via atropine. These results suggest that the QTVI may reflect both cardiac sympathetic and parasympathetic activity in those with incomplete SCI, depending on the autonomic state of the individual. Results from **Study 2** demonstrate high day-to-day reproducibility of the QTVI in participants with SCI, regardless of injury level, and also in those with high level injuries who may have reduced cardiac sympathetic regulation. Results from **Study 3** showed that indices of cardiac parasympathetic activity (HRV and QTVI) were correlated with left ventricular filling in able-bodied individuals but not in individuals with SCI, suggesting a disconnect between cardiac parasympathetic activity and diastolic function after SCI. In addition, for the able-bodied group, the cold face test (CFT) increased vagal activity which was associated with bradycardia and augmentation of diastolic filling. However, for the SCI group, the increase in vagal activity during the CFT was associated with no change in heart rate and an attenuation of diastolic function. Results from **Study 4** showed that although individuals with SCI were hypovolemic, resting diastolic velocities were similar compared to the able-bodied group. This

maintenance of diastolic function appeared to be attributed to the cardiac atrophy that manifests following SCI. In addition, there was no between-group difference in the diastolic response to rapid saline infusion.

Findings from this dissertation suggest that the QTVI is a valid and reliable tool for non-invasively estimating cardiac autonomic regulation in individuals with SCI. In addition, although the mechanical aspect of diastolic function is preserved after SCI, atypical vagal-diastolic interactions may impair ventricular filling in this population.

**Acknowledgements**

This PhD thesis is the product of 4 years of hard word dedicated to improving the quality of life of individuals with spinal cord injury. I could not have produced this work or reached this stage of my academic career without the continuous help and support of certain individuals that I would like to mention.

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**List of Abbreviations**

| | |
|---|---|
| **A** | Late transmitral flow velocity |
| **A'** | Late myocardial diastolic velocity |
| **AIS** | ASIA (American Spinal Injury Association) Impairment Scale |
| **CFT** | Cold face test |
| **CV** | Cardiovascular |
| **DT** | Deceleration Time |
| **E** | Early transmitral flow velocity |
| **E:E'** | Left ventricular filling pressure |
| **E'** | Early myocardial diastolic velocity |
| **E:A Ratio** | Ratio of early to late transmitral flow velocity |
| **ECG** | Electrocardriogram |
| **HF** | High frequency component of heart rate variability |
| **HR** | Hear rate |
| **HRV** | Heart rate variability |
| **IVRT** | Isovolumetric relaxation time |
| **LF** | Low frequency component of heart rate variability |
| **LV** | Left ventricle/left ventricular |
| **PNS** | Parasympathetic nervous system |
| **QTm** | QT-interval mean |
| **QTv** | QT-interval variability |
| **QTVI** | QT-variability index |
| **RMSSD** | Root mean square of differences between RR intervals |
| **RRm** | RR-interval mean |
| **RRv** | RR-interval variability |
| **SCI** | Spinal cord injury |
| **SDNN** | Standard deviation of all RR intervals |

| | |
|---|---|
| **SNS** | Sympathetic nervous system |
| **TD** | Tissue Doppler |

## Chapter 1 – Introduction

The heart is arguably the most dynamic muscle in the body, which can be attributed to its intricate anatomical muscle fibre orientation, its ability to contract (and relax) in various directional planes of motion and the cascade of biochemical, electrical and mechanical events that are continuously occurring within it. In addition, cardiac structure and function are constantly influenced by a multitude of external factors such as vascular compliance, hemodynamic load, inflammation and neuro-hormonal activation[1]. All of these aforementioned factors are required to operate synergistically in order for the heart to function in an appropriate manner. Although the cardiovascular system is a combination of various physiological systems, it still operates as one entity via positive and negative feedback loops. As such, altered activity in any of the stated factors can result in cardiac maladaptation. It is important to note that "cardiac system" refers to the heart *per se*, whereas the "cardiovascular system" refers to the heart and vasculature.

Spinal cord injury (SCI) is a condition that typically leads to dysfunctions in most physiological systems in the body. These dysfunctions can be a direct consequence of the SCI itself, as is the case for motor and sensory paralysis, or they can be due to the symptoms arising from SCI, such as pressure ulcers and metabolic disorders. This thesis however, focuses on the cardiac system and how it is influenced by SCI. More specifically, this thesis explores two aspects of the cardiac system following SCI; the first being cardiac autonomic function and the second being diastolic function. In addition, the interaction between autonomic and diastolic function in those with SCI will also be examined.

Regarding cardiac autonomic activity, the heart is normally under the constant influence of both the parasympathetic and sympathetic nervous systems[2]. However, due to spinal damage following SCI, there is a loss, or a reduction, of supraspinal control of the heart, thus attenuating cardiac autonomic regulation. Accordingly, an important question in the field of SCI research is: Is there a valid, reliable and non-invasive tool that will allow us to measure the amount of preserved cardiac autonomic function in humans with SCI? Having such a tool would be of substantial clinical value, as it could improve our ability to diagnose and track autonomic dysfunctions after SCI. This tool could also be used for the assessment of cardiovascular risk, and it could allow researchers to monitor autonomic responses to treatments and interventions. The electrocardiogram (ECG) is a non-invasive tool that reflects global cardiac electrical activity, and since cardiac electrical activity is highly modulated by autonomic activity, the ECG may be used as an indirect index of autonomic function. Traditional ECG-based measures of cardiac autonomic activity include frequency and time-domain measures of beat-to-beat temporal variability, known as heart rate variability (HRV). However, certain aspects of HRV have their limitations, as the physiological basis of some HRV parameters do not necessarily correlate with cardiac autonomic activity in able-bodied individuals[3] or those with SCI[4]. The QT-variability index (QTVI) is a relatively new, ECG-based, measure of beat-to-beat repolarization variability. Recent animal studies have demonstrated correlations between the QTVI and cardiac sympathetic and parasympathetic outflow[5]. However, the relationship between the QTVI and cardiac autonomic activity in humans remains unclear and warrants further investigation as it may hold promise as a means of gauging cardiac autonomic function in individuals with SCI. Therefore, the first two manuscripts of this thesis will focus on

examining the validity and reliability of the QTVI as a measure of cardiac autonomic activity in those with SCI.

To date, findings regarding left ventricular diastolic function following SCI are equivocal, as some studies have shown reduced diastolic function[6,7] whereas others showed that it is preserved[8,9]. From a cardiac pathophysiological perspective, impaired diastolic function suggests an increase in left ventricular stiffness and reduced compliance due to fibrosis, which impairs the ventricle's ability to rapidly fill with blood, and causes an elevation in ventricular filling pressure for a given blood volume[10]. However, individuals with SCI do not typically display co-morbidities that lead to increased myocardial fibrosis, such as hypertension. Therefore, it is quite possible that the purported impairments in diastolic function following SCI are not a result of fibrotic or diseased ventricles, but could be due to other systemic changes that take place after SCI, specifically changes in cardiac parasympathetic activity and reduced preload.

Experimental studies have shown, via both vagal stimulation[13] and withdrawal[14], that parasympathetic activity may play a role in modulating diastolic function. Specifically, increased vagal activity may improve ventricular filling. In support of these findings, diastolic impairments have been reported to be more severe in individuals with lower cardiac parasympathetic outflow in both healthy[11] and diseased[12] able-bodied populations. Furthermore, diastolic function is highly influenced by preload as an increase in venous return results in elevated left atrial filling, thus increasing atrial pressure and resulting in higher diastolic filling velocities. Alterations in both these factors (parasympathetic activity and preload) are typically manifested after SCI, such that a reduction in parasympathetic activity (due to physical inactivity), and reduced preload (due to hypovolemia and an

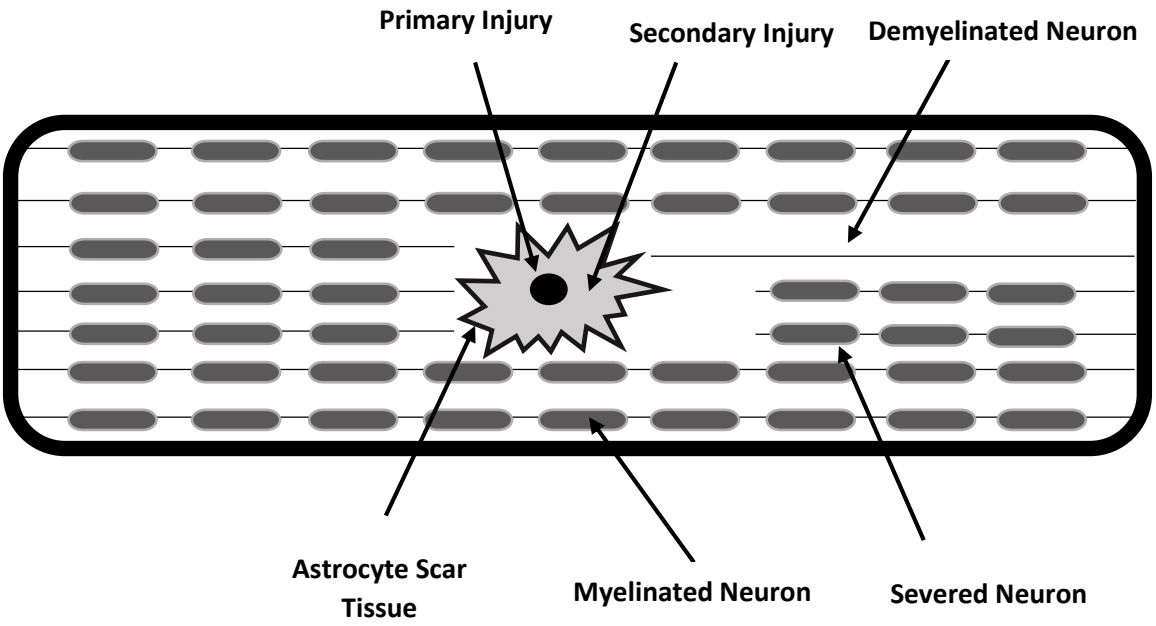
ablation of the muscle pump effect) are typically observed. Accordingly, it is quite possible that the putative diastolic impairments in those with SCI are a result of either a reduced cardiac vagal activity or a reduced preload, or a combination of the two. Therefore, the last two manuscripts of this thesis will examine the relationship between cardiac vagal activity and diastolic function, as well as the influence of preload on diastolic function, in individuals with SCI.

## Chapter 2 – Literature Review

### Etiology of Spinal Cord Injury

SPINAL CORD INJURY (SCI) is a particularly abrupt and life altering neuromuscular condition. It can have detrimental physiological, psychological and social impacts on the individual. The main pathology of SCI involves death of neural tissue within the spinal cord, thus obscuring supraspinal signals from reaching their target organs to perform normal bodily functions. This can result in motor paralysis, autonomic dysfunctions and sensory malfunction. Some of these dysfunctions manifest immediately after an injury, while others develop chronically over time. Spinal cord tissue damage can occur from traumatic or non-traumatic causes. Traumatic SCI occurs when an external physical impact damages the spinal cord either directly, or by crushing the vertebral column around the spinal cord or by reducing blood supply to the spinal cord. In contrast, non-traumatic SCI can result from infection, disease or radiation that causes neural tissue necrosis. The resultant spinal cord damage from the initial injury is called the primary injury, which leads to localized and often small spinal damage. However, within hours to approximately a month after the initial trauma, the secondary injury phase occurs, whereby the site of injury starts to expand due to inflammation, ischemia and ion degradation (See Figure 1).

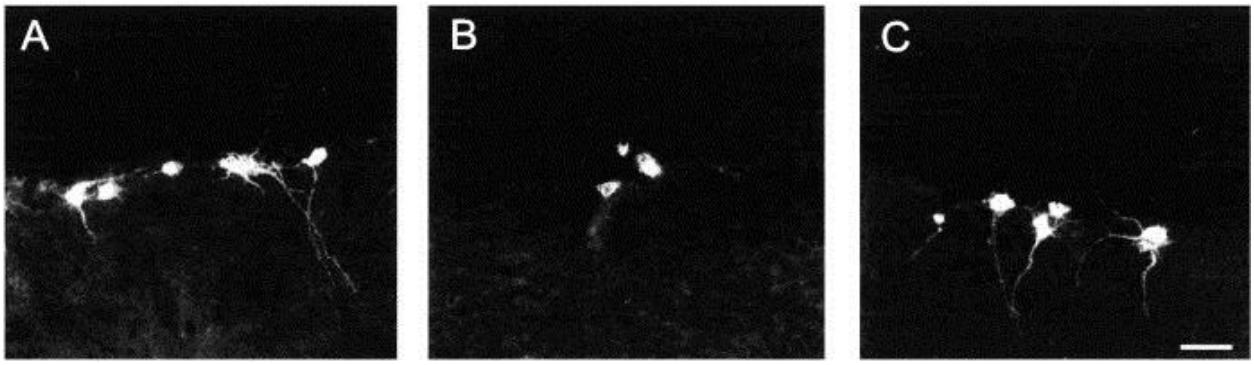
**Figure 1: Spinal Cord Injury**




It is the secondary damage that causes the majority of complications following an injury due to the increased neural tissue death. Immediately after a SCI, the individual experiences both spinal shock and neurogenic shock. Spinal shock is a temporary period of complete motor, and often sensory, paralysis below the level of injury. This period lasts for approximately a week until the individual starts to regain some function, which may be attributed to spontaneous regeneration or myelination of neural tissue. Neurogenic shock on the other hand is complete ablation of autonomic regulation, which is characterized by dangerously low blood pressure and uncontrolled cardiac arrhythmias. Neurogenic shock also dissipates within approximately a week after an injury, however, this is the time period when other autonomic dysfunctions, such as autonomic dysreflexia, start to manifest. Animal studies have shown that axons undergo severe atrophy during the neurogenic shock stage, but regain their size and arborisation at approximately the same time as when autonomic regulation starts to work again. As axonal size and arborisation is regained, there

is a concomitant manifestation of autonomic dysreflexia however, suggesting inappropriate neural connections are formed after the neurogenic shock period[15].

**Figure 2: Axonal Morphology before and after Injury**




A: Normal axonal size and arborisation at baseline; B: Axonal atrophy and loss of arborisation immediately after SCI; C: Axonal size and arborisation regained after 4 weeks of SCI, concomitant with autonomic dysreflexia.

**Classifications of Spinal Cord Injury**

Spinal cord injury is classified according to three categories: level of injury, severity of injury and whether the injury is an upper or a lower motor neuron injury. Regarding level of injury, SCI that results in damage to the cervical spine (above T1) is called tetraplegia, which involves impaired sensory and/motor function in the upper limbs, trunk, pelvic area and lower limbs. An injury that inflicts damage below the cervical region is known as paraplegia, which involves impaired sensory and/motor function in the trunk, pelvic area and lower limbs. The severity of SCI is governed by its completeness. Anatomically, a complete SCI spans the whole width of the spinal cord thus not allowing any spared neurons to bypass the site of injury. An incomplete injury does not span the whole width of the spinal cord, resulting in some neurons to bypass the injury. With respect

to function, in a complete injury there is no sensory or motor function below the neurological level of injury, including the 4th and 5th sacral dermatomes and myotomes. In an incomplete injury, there is spared motor and/or sensory function below the neurological level of injury, including the 4th and 5th sacral dermatomes and myotomes. A more accurate way to assess the level of completeness is by the ASIA (American Spinal Injury Association) impairment scale (AIS) (Appendix A). In order to assign an individual with an AIS score, the neurological level of injury needs to be established (See Appendix B) using motor (See Appendix C) and sensory (See Appendix D) examinations. Finally, an upper motor neuron injury refers to an injury to the central nervous system (spinal cord) whereas a lower motor neuron injury refers to an injury to the peripheral nerves.

**Epidemiology of Spinal Cord Injury**

The incidence rate of SCI in Canada is estimated to be approximately 4259 new cases each year. Of this, an estimated 42% (1785) are the result of traumatic SCI, whereas 58% are from non-traumatic causes. The prevalence rate of SCI in Canada is estimated to be 85,556 individuals living with SCI. Of this total, 51% (43,974) are the result of traumatic causes and 49% are from non-traumatic incidents[16]. Most injuries are acquired during young adulthood, between ages of 18-29 and are mostly due to motor vehicle accidents, sports or violence[17]. A second peak of acquired injuries takes place during old age (70 years and older) due to falls[17]. In Canada, the incidence of injuries according to spinal segments are as follows: 29% cervical, 21% thoracic and 50% lumbosacral[18].

## Autonomic Control of the Cardiovascular System

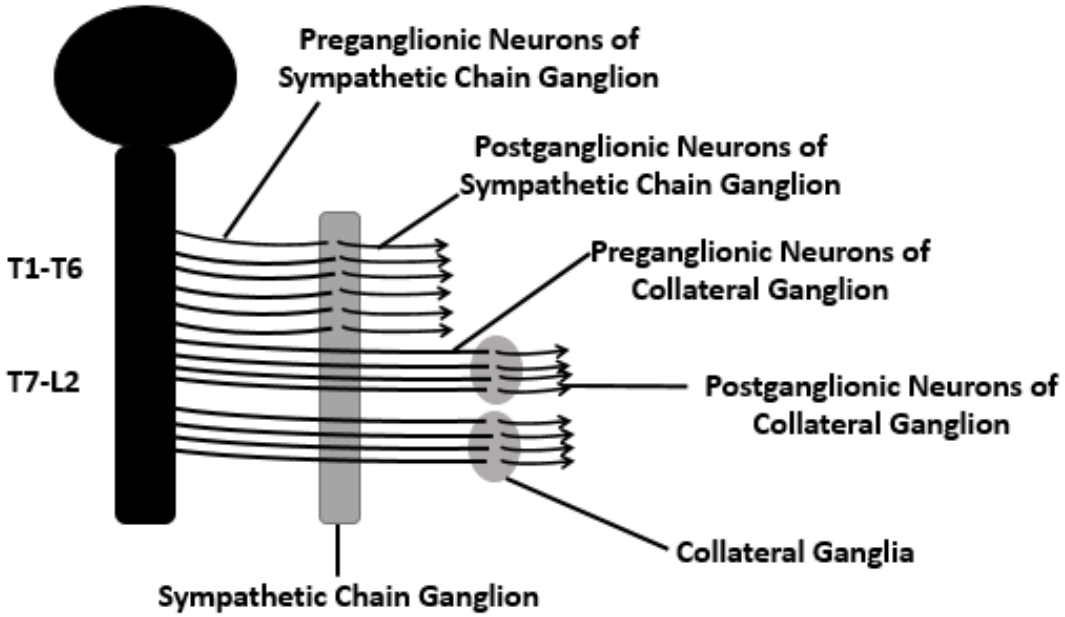
Normal autonomic control of the cardiovascular (CV) system is executed through the constant balance of the sympathetic (SNS) and parasympathetic nervous systems (PNS)[2]. In general, both systems operate in opposing manners, the SNS is involved in the fight or flight response, such as increasing heart rate (HR), cardiac contractility and blood pressure. In contrast, the PNS is involved in maintaining resting state, such as reducing HR. The efferent pathways of both the SNS and PNS originate in the central nervous system, where neural messages pass from the preganglionic neurons through the autonomic ganglia, towards the post ganglionic neurons which then reach the target organ.

## Sympathetic Cardiovascular Function

The neuroanatomy of cardiovascular sympathetic control consists of short sympathetic preganglionic neurons that originate in the lateral horn of the grey matter and exit the spinal cord through the thoracic and upper lumber spinal segments (T1-L2)[19]. The sympathetic preganglionic neurons between T1-T6 synapse with the sympathetic chain ganglia which then synapse with the long postganglionic neurons. The post ganglionic neurons release catecholamines into the effector organ, being the heart in this case in order to increase HR and ventricular contractility. Therefore, cardiac sympathetic activity is regulated via sympathetic spinal nerves of T1-T6[20]. Sympathetic preganglionic nerves below the T6 spinal segment bypass the sympathetic chain and synapse with collateral ganglia, which then synapse with postganglionic neurons of the abdomen and pelvis in order to form the splanchnic nerves. Therefore, sympathetic spinal nerves

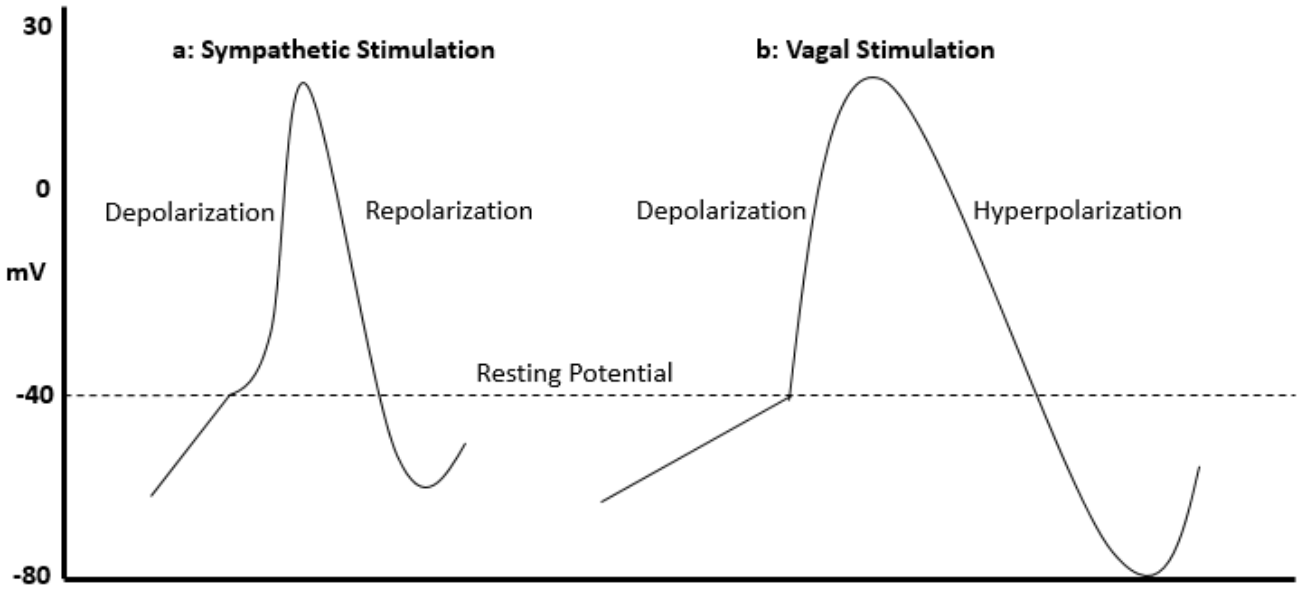
between T7-L2 play a critical role in regulating blood pressure though their influence on the splanchnic vascular bed[19] (See Figure 3).

**Figure 3: Cardiovascular Sympathetic Regulation**




The SNS mainly exerts its effects through the catecholamines epinephrine and norepinephrine. In order to increase HR and ventricular contractility, signals from supraspinal structures such as the rostural ventral medulla and raphe padillus relay excitatory signals to the dorsomedial hypothalamus. These supraspinal signals will descend via the brainstem down the spinal cord and exit from T1-T6, where the corresponding postganglionic neurons will release norepinephrine into the sino-atrial node in the right atrium[19]. Norepinephrine will bind onto β-1 adrenoceptors in the sino-atrial node membrane which will increase the rate of early depolarization, causing the threshold membrane to be reached faster, which creates more rapid action potentials and thus increasing HR (See Figure 4).

**Figure 4: Influence of Autonomic Activity on Rate of Action Potential Generation**




a: rapid rate of early depolarization from sympathetic activity on the sino-atrial node; b: slow rate of early depolarization and hyperpolarization from vagal influence on sino-atrial node.

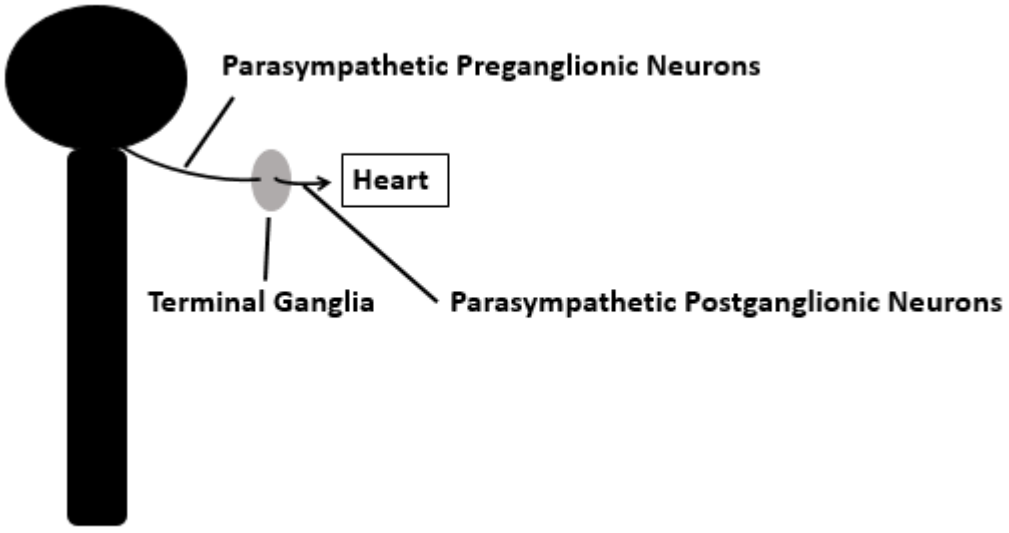
In addition, norepinephrine released from the T1-T6 post ganglionic neurons will bind directly onto the ventricular walls, causing an increase in calcium release, thereby forming more actin and myosin cross-bridges, which therefore increases myocardial contractility[2]. Furthermore, in order to increase blood pressure, the sympathetic postganglionic neurons between T7-L2 will release norepinephrine, which will bind onto the alpha-1 adrenoceptors of blood vessels in the splanchnic bed, causing smooth muscle contractions thereby elevating pressure.

**Parasympathetic Cardiovascular Function**

Cardiac parasympathetic activity is regulated through the vagus nerve (Cranial Nerve X). Unlike sympathetic nerves, the parasympathetic preganglionic neurons are long

and originate in the dorsal motor nucleus of the medulla oblongata and exit from the brainstem as the vagus nerve. The parasympathetic preganglionic neurons synapse with the terminal ganglia, which is connected to the short postganglionic neurons. The postganglionic neurons will exert their effect onto the effector organ, the heart, via the neurotransmitter acetylcholine (See Figure 5).

**Figure 5: Cardiac Vagal Regulation**




In order to reduce HR, acetylcholine will bind onto the β-1 receptors of the sino-atrial node, which will cause hyperpolarization and will slow down the early depolarization phase (See Figure 4). Through these mechanisms, the threshold potential is reached at a slower rate, thus resulting in slower generation of action potentials which therefore, results in a slower HR[22].

**Cardiovascular Autonomic Regulations after Spinal Cord Injury**

The severity of CV autonomic dysfunctions following SCI has been shown to be directly related to the level and completeness of the injury. As mentioned above, cardiac sympathetic function is regulated by spinal nerves between T1-T6, and caudal to that are the sympathetic nerves responsible for blood pressure regulation[19]. Therefore, cervical injuries, or high thoracic injuries, may result in the loss, or attenuation, of supraspinal regulation of HR and blood pressure. In addition, the attenuation of supraspinal regulation over the splanchnic bed can result in hypotension[22], orthostatic hypotension[23], and potentially dangerous reflexive fluctuations in blood pressure known as autonomic dysreflexia[24]. In contrast, cardiac parasympathetic activity is not directly affected by the SCI, as the vagus nerve bypasses the spinal cord, and therefore cardiac parasympathetic innervation of the heart remains intact[25]. Such an imbalance in cardiac sympathetic and parasympathetic activity, however is associated with an increased risk of cardiac arrhythmias in individuals with SCI[22]. Thus, higher level injuries, specifically those above T1, are associated with a greater risk for cardiovascular autonomic dysfunction.

Moreover, more complete injuries result in greater and more severe CV autonomic complications[19]. Histopathological examination of post-mortem spinal cord tissue showed that individuals with tetraplegia, who had severe CV abnormalities such as bradycardia, hypotension and autonomic dysreflexia, demonstrated more axonal degeneration, specifically white matter, compared to those with no or minor CV abnormalities[26].

## Electrocardiographic Measures of Cardiac Autonomic Activity

The electrocardiogram (ECG) provides an easy and non-invasive means of measuring the electrical activity of the heart. Since cardiac electrophysiology and beat-to-beat control of HR are tightly regulated by the autonomic nervous system[27,28,29], ECG can be employed as an indirect index for assessing cardiac autonomic function. This section will discuss two ECG-based measures of cardiac autonomic function: heart rate variability (HRV) and the QT-variability index (QTVI).

### Heart Rate Variability

Heart rate variability is an ECG-based measure of cardiac autonomic control that takes advantage of the normally occurring oscillations in beat-to-beat variability[30]. On a physiological basis, the parasympathetic system exerts its effects on the heart by releasing the neurotransmitter acetylcholine from the parasympathetic postganglionic neurons into the sino-atrial node, which decreases HR quickly (latencies to onset of 50-100 ms) and is also rapidly hydrolyzed by cholinesterase[21,31]. Therefore, due to the rapid activity of vagal influence on the heart, the parasympathetic system exerts its effects HR on a beat-to-beat basis and thus, beat-to-beat temporal variability can be accounted for by parasympathetic activity. In contrast, the sympathetic system exerts its effects on the heart mainly through the hormone norepinephrine, which is released by the sympathetic postganglionic neurons (T1-T6) into the sino-atrial node. Unlike acetylcholine, norepinephrine exerts its effects to increase HR in a slow manner (1-3 seconds latency) but is also terminated slowly by the presynaptic reuptake and diffusion into the

surrounding extracellular fluids[21,31]. Therefore, due to the slow effects of the sympathetic system, it cannot influence HR on a beat-to-beat basis.

Heart rate variability can be analyzed via time domain or frequency domain techniques. Time domain analysis of HRV involves mathematically computing the normal temporal variability that occurs between a given number of beats, some of these include standard deviation of all RR intervals (SDNN), root mean square of differences between RR intervals (RMSSD)[32], and the coefficient of variance of a given number of RR intervals. Since time domain measures of HRV directly assess the amount of beat-to-beat variability, they are believed to mainly reflect cardiac parasympathetic activity with some influence from sympathetic activity. Frequency domain analysis on the other hand is believed to provide a measure of both sympathetic and parasympathetic autonomic activity to the heart. In brief, successive RR intervals from an ECG (See Figure 6a) are plotted against time to create a tachogram (See Figure 6b) which is then analyzed through spectral density analysis. In resting healthy humans, successive RR intervals oscillate around 2 main frequencies: low frequency (LF; 0.04-0.15 Hz; centre frequency: 0.1 Hz) and high frequency (HF; 0.15-0.40 Hz; centre frequency: 0.25 Hz)[33]. The relative predominance, or power, of the LF and HF oscillations are then quantified and graphically represented in a power spectrum (See Figure 6c).

**Figure 6a: Consecutive RR intervals on an ECG**


**Figure 6b: Tachogram of RR intervals**

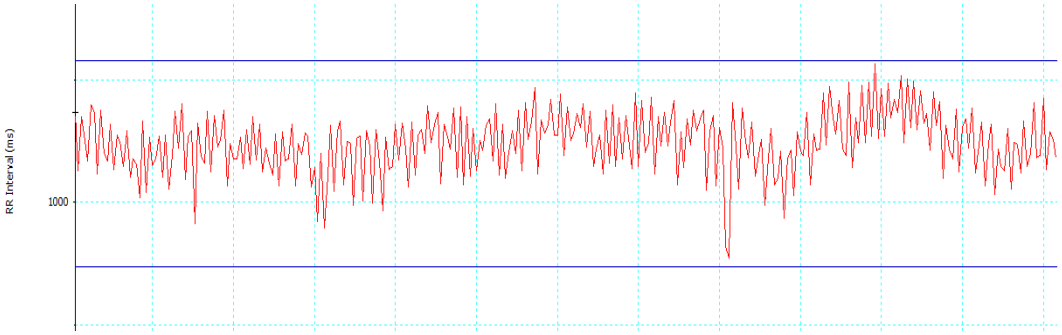
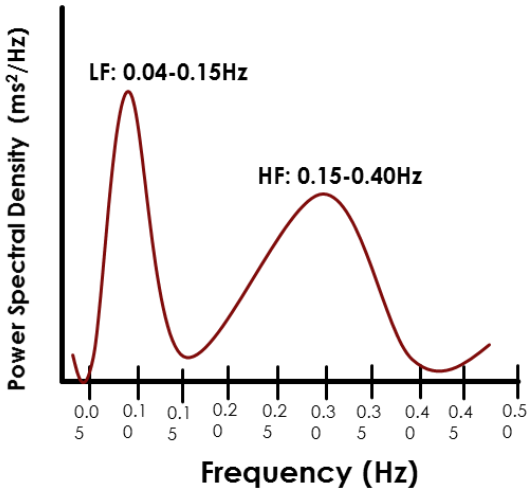



**Figure 6c: Spectral high and low frequency of RR intervals**




a: Successive R-R intervals from an ECG recording; b: tachogram of the ECG recording; c: depiction of low frequency and high frequency ranges in healthy individuals.

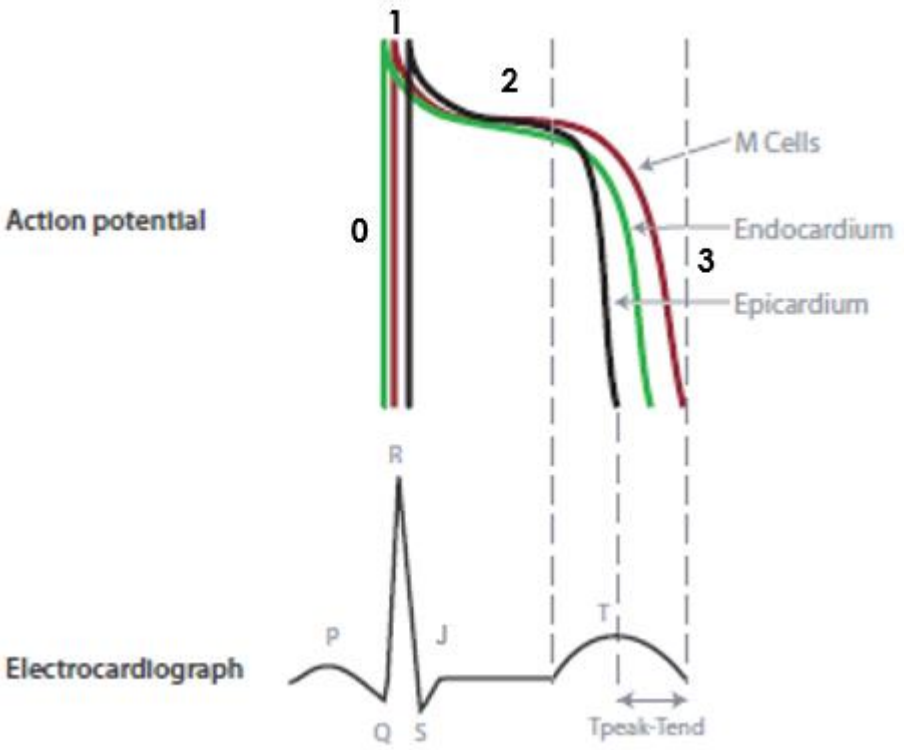
The HF power portion of HRV reflects cardiac parasympathetic outflow as evidenced by its complete ablation after cholinergic blockade[34,35] and following parasympathectomy[36]. The consensus on the physiological basis of LF power however remains debatable. It is generally considered that LF power reflects both autonomic limbs of the nervous system, since it has been shown to decrease in response to both cholinergic and β-blockers[34]. In addition, LF power was demonstrated to decrease by 50% after parasympathectomy, and an additional 20% reduction following administration of selective β-blocker[36]. However, others consider LF to be a poor marker of cardiac sympathetic activity, as LF has been shown to decrease after experimental coronary artery occlusion[37] and after inducing congestive heart failure[38], both of which typically result in elevated sympathetic activity.

**QT-Variability Index**

The QT interval of the ECG represents the duration of global myocardial repolarization. From an electrophysiological perspective, the QT interval temporally corresponds with the action potentials of all three myocardial layers. As shown in Appendix L, the endocardial layer is the first to repolarize in Phase 1, which takes place approximately at the same time as the Q-wave. The initial repolarization in Phase 1 is a result of the transient outward potassium current, however, the cell potential is maintained and plateaus during Phase 2 because of the inward calcium current. Phase 3 represents myocardial repolarization which is driven by the outward movement of potassium ions carried by the rapid and slow delayed rectifier potassium currents. The sum of the transmural cardiac repolarization is represented by the T wave on the ECG. The initial ascending limb of the T wave is due to an increase in the transmural dispersion

between the ventricular layers, where an increase in the gradient results in an increase in electrical voltage. The epicardium is the first to fully repolarize, which corresponds with the T-peak on the ECG, while the mid myocardium is the last to repolarize, thus coinciding with the T-wave terminus[39,40] (See Figure 7).

**Figure 7: Myocardial Repolarization and Electrocardiographic Activity.**




Phase 1: initial myocardial repolarization resulting from initial transient outward potassium movement which coincides with the J wave; Phase 2: myocardial repolarization plateau characterized by outward potassium movement and inward calcium influx; Phase 3: myocardial repolarization from transient outward potassium movement. Epicardial repolarization coincides with the T-wave peak and Mid myocardial repolarization coincides with the T-wave terminus.

Similar to the RR interval, the QT interval displays a degree of variability on a beat-to-beat basis. Such beat-to-beat repolarization variability can be calculated via the QTVI according the formula developed by Berger et al. (1997):

$$QTVI = Log_{10} [(QTv/QTm^2) / (RRv/RRm^2)]$$

where, QTv is the QT interval variance, $QTm^2$ is the mean QT interval squared, RRv is the RR interval variance and $RRm^2$ is the mean RR interval squared[41]. Thus, QTVI values increase either due to an elevation in QT interval variance (numerator) or from a decrease in RR interval variance (denominator). Since both the QT and RR intervals are influenced by cardiac autonomic activity[27,34,42], the QTVI may be a superior method of gauging cardiac autonomic activity compared to HRV, as it accounts for both intervals in its calculation. Experimental studies in healthy humans have shown that the QTVI increases when sympathetic activity is elevated, as demonstrated by CV stress maneuvers and infusion of the β-adrenoceptor agonist isoprotenerol[43]. Clinical studies have also shown that the QTVI is elevated in patients with conditions associated with elevated cardiac sympathetic activity such as hypertension[44], heart failure[45], dilated cardiomyopathy[41] and anxiety[46]. These findings suggest that QTVI may be influenced by cardiac sympathetic activity. However, other investigators have also shown that the QTVI is elevated in situations that are associated with reduced vagal activity, such as healthy aging[47] and cardiac autonomic neuropathy[48]. The most compelling evidence for the influence of cardiac autonomic activity on the QTVI was from Piccirillo et al. (2009), who showed that during healthy resting conditions, QTVI was inversely related to integrated vagal nerve activity in dogs ($r=-0.47$; $p=0.01$). However, after ventricular pacing induced heart failure, the QTVI was directly associated with left stellate

ganglionic activity (r=0.57; p=0.01)[5]. This provides strong evidence that the QTVI is associated with cardiac sympathetic outflow during times of autonomic arousal, and is also inversely reflective of cardiac vagal outflow during resting conditions. In other words, it may reflect both autonomic limbs depending on the state the subject is in.

**Heart Rate Variability in Spinal Cord Injury**

Heart rate variability has been employed for over two decades for the assessment of cardiac autonomic function in the SCI population. Although HRV has been shown to be reliable and reproducible in individuals with SCI[49], the physiological basis of this measure remains somewhat equivocal, especially pertaining to the interpretation of the LF component. For example, earlier work by Inoue et al. (1990) suggested that LF power corresponds to cardiac sympathetic outflow, as individuals with complete tetraplegia failed to show any sign of it[50]. Contradicting work by Koh et al. (1994) demonstrated that LF power reflects cardiac vagal outflow, as atropine administration abolished LF power in individuals with SCI[51]. Guzzetti and colleagues (1994) showed that half of their cohort with complete tetraplegia exhibited a detectable LF component, suggesting that in some individuals, LF power corresponds to sympathetic spinal rhythmicity[52]. Moreover, Inoue et al. (1995) reported that the physiological correlates of LF power in individuals with tetraplegia may be different from that in able-bodied individuals, and that LF power in individuals with SCI is simply a reflection of reflex sympathetic outflow caused by stimuli from the periphery (bladder or bowel distension or spasms from the limbs)[53]. In conclusion, these conflicting results certainly lack a consensus on the physiological basis of LF power in individuals with SCI and therefore, this variable should be employed and interpreted with caution. High frequency power on the other hand has been shown to

reflect cardiac parasympathetic activity in individuals with SCI. In a recent study, Cotie et al. (2010) demonstrated a 99% ablation of HF power in individuals with SCI following atropine administration[35].

Although HF power reflects cardiac vagal outflow, reports have shown that it is reduced in individuals with SCI, suggesting attenuation of cardiac parasympathetic activity. This has been attributed to the body's attempt to maintain cardiac sympathovagal modulation[54,55]. However, other investigators speculate that the reduction in HF power may be due to altered cholinergic receptor response to acetylcholine, as individuals with SCI demonstrate a blunted HR response to vagal withdrawal[56]. The same group also showed that compared to able-bodied individuals, those with SCI demonstrate less of an increase in HF power and a paradoxical increase in HR in response to the cold face test[57]. These observations suggest that a possible alteration in sino-atrial nodal response to vagal activity could be accountable for the reduced HF power reported following SCI. However, another possible explanation for the reduced HF power following SCI is the profound physical inactivity associated with this population. This is evidenced by Ditor et al. (2005b) who reported an increase in HF following an exercise program in individuals with SCI[58]. In addition, other studies have shown that physically fit SCI individuals have better HF dynamics compared to their sedentary counterparts, as shown by higher resting HF[59] and faster post-exercise vagal recovery[60].

**QT Variability Index in Spinal Cord Injury**

Because the QTVI is a relatively novel index of cardiac autonomic modulation, there is a paucity of literature on this method in individuals with SCI. In the first study to investigate the QTVI in individuals with SCI, La Fountaine et al. (2010) compared

resting QTVI values between able-bodied controls and individuals with tetraplegia (C3-C8; gross cardiac sympathetic dysfunction), high paraplegia (T1-T6; variable cardiac sympathetic dysfunction) and low paraplegia (T7-L5; cardiac sympathetic function intact)[61]. Results showed that all SCI groups had significantly elevated QTVI values compared to the able-bodied group (tetraplegia: -0.68±0.17; high paraplegia: -0.63±1.32; low paraplegia: -0.65±0.99; able-bodied: -1.34±1.03; p=0.01) while there were no differences between any of the SCI groups. In addition, QTVI was negatively correlated with HF power in all SCI groups, which is in agreement with the previously mentioned observations from Piccirillo et al. (2009) suggesting that QTVI is elevated when vagal outflow is reduced[5]. Further, Ravensbergen et al. (2012) compared QTVI values between able-bodied controls and individuals with SCI who were further subdivided into groups based on 1) region of injury (cervical or thoracic), 2) level of injury (below or above/at T5), 3) autonomic completeness (complete or incomplete), and 4) motor and sensory completeness (complete or incomplete)[62]. Similar to La Fountaine et al. (2010), the QTVI was significantly higher in all SCI individuals compared to the able-bodied group (-1.03 ± 0.100 vs. -1.31 ± 0.078; p= 0.019). Further analysis showed that QTVI values were related to the level of injury and autonomic completeness, as those with lesions above T5 and those with autonomically complete injuries had significantly higher QTVI compared to able-bodied controls[62].

**Left Ventricular Functional Anatomy**

The left ventricle (LV) is the thickest wall in the heart. It is located posteriorly and laterally to the right atrium and forms the apex of the heart. Its extra thick walls allow for the development of high enough pressures during contraction to eject blood

throughout the whole body. The LV is composed of three superimposed but interconnected layers, the epicardium, mid myocardium and endocardium. The different layers of the LV are arranged in a spiral/helical manner, resulting in counter directional twisting, providing an efficient distribution of regional stresses and strain[63]. When examining the LV transmurally, the muscle fibre direction is predominantly longitudinal in the endocardial layer, transitioning into a circumferential direction in the mid myocardium and then going back to being longitudinal in the epicardial surface[64]. In addition, the epicardium is oriented in a left helical plane (See Figure 8a) whereas the endocardial fibers are oriented in a right helical direction (See Figure 8b). The different muscle fibre orientation throughout the LV wall has functional implications that optimize contraction and relaxation.

**Figure 8a: Cardiac Epicardial Layer**

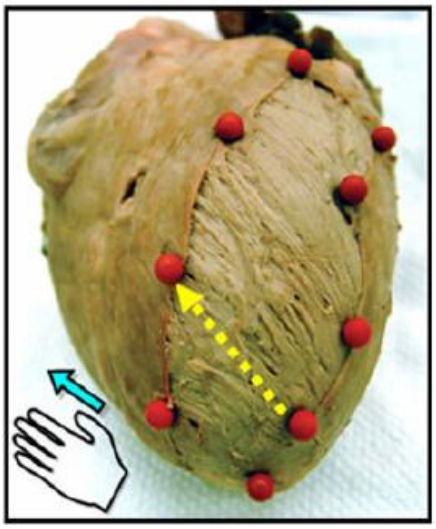


Figure depicts a left helical muscle fibre orientation. Figure from Sengupta et al. (2007)[64].

**Figure 8b: Cardiac Endocardial Layer**

Figure depicts right helical muscle fibre orientation. Figure from Sengupta et al. (2007)[64].

Initially, the rapid spread of electrical activity during depolarization spreads in an apico-basal direction within the subendocardium, which initiates the contraction process. Following the spread of action potentials, the subendocardium shortens in a right-handed helical orientation, which results in a rapid build-up of intraventricular pressure, coinciding with the isovolumetric contraction period. This subendocardial shortening causes a simultaneous subepicardial fibre stretch which allows the muscle to optimize the length-tension relationship required for the subsequent contraction. During ejection, both the subendocardial and subepicardial layers contract in opposing spiral directions. Contraction force in the LV apex exceeds the contraction force in the base, causing the contractile to move in an apico-basal direction thus milking blood out of the LV when the intraventricular pressure exceeds that of the aorta[64].

**Left Ventricular Diastolic Function**

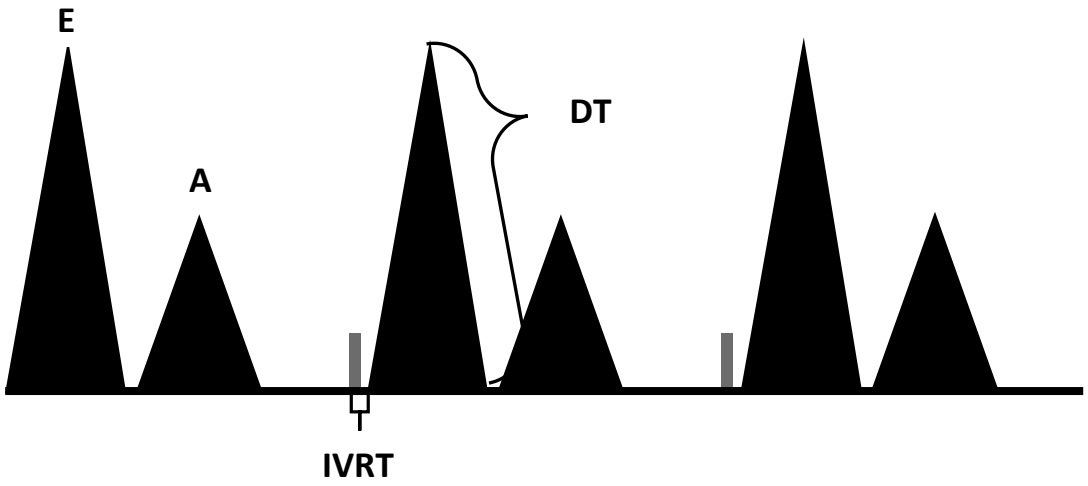
Left ventricular diastole involves 3 stages: isovolumetric relaxation, early diastole and late diastole. Isovolumetric relaxation begins before aortic valve closure at the end of systole, which involves subendocardial untwisting in an apico-basal direction. The isovolumetric relaxation period makes up 40% of LV diastole and is the most critical period for normal LV relaxation to occur because it results in a rapid reduction in intraventricular pressure. Such a rapid reduction in intraventricular pressure creates a pressure gradient between the LV and left atrium which facilitates early passive LV filling when atrial pressure exceeds that of the LV. Early diastole is marked by mitral valve opening and rapid passive filling of the LV due to the pressure gradient formed during the isovolumetric period. In addition, the LV apex rapidly untwists during early diastole, creating a suction force which further augments the rapid movement of blood

from the left atria into the LV. At the end of early diastole, LV pressure exceeds that of the left atria due to the increased ventricular volume, which marks the end of early diastole. However, there remains a small amount of blood in the left atria, therefore, the left atria will contract and empty the remaining blood in it into the LV, marking late diastole. In contrast to early diastole, late diastole is an active process (atrial contraction) and the velocity of LV filling is much slower.

**Echocardiographic Assessment of Diastolic Function**

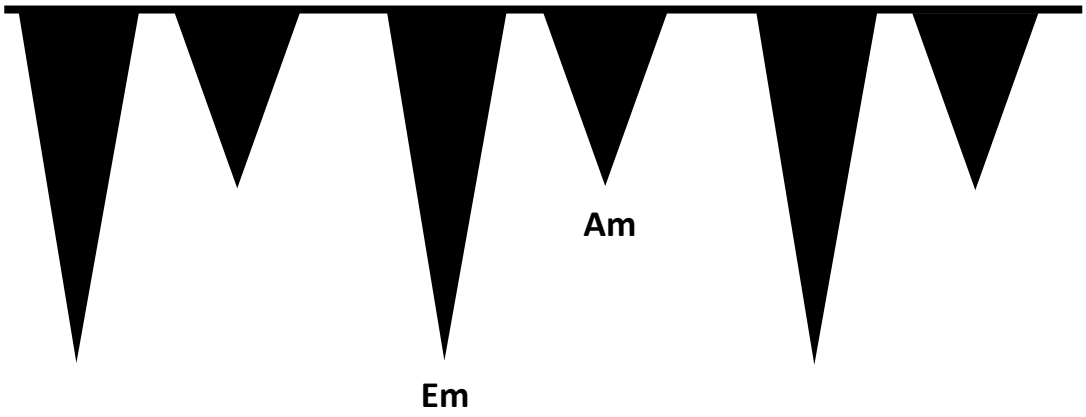
Echocardiography employs ultrasound technology to assess LV diastolic function. This can be done via pulsed-wave Doppler or tissue Doppler (TD) techniques. Pulsed-wave Doppler assesses the velocity of blood moving through the mitral valves during early and late diastole, whereas TD assesses the velocity of myocardial tissue during early and late diastole. From the pulsed-wave Doppler technique, early (E) and late (A) tranmsitral flow velocities can be measured as well as their ratio (E:A). Under normal conditions, most of the blood will fill the LV during early passive diastole, therefore, the E velocity is typically greater than A velocity resulting in an E:A ratio greater than 1. The deceleration time (DT) of early ventricular filling can also be assessed from the E transmitral wave. In addition, the isovolumetric relaxation time (IVRT) can be assessed as the time interval between aortic valve closure and the onset of ventricular filling (See Figure 9). As mentioned above, the TD technique examines myocardial tissue velocity during diastole. Peak early (Em) and late (Am) myocardial diastolic velocities can be assessed from the basal and septal annular segments of the LV (See Figure 10).

**Figure 9: Pulse-Wave Doppler Measurement of Diastole**




E: early transmitral flow velocity; A: late transmitral flow velocity; IVRT: isovolumetric relaxation time; DT: deceleration time.

**Figure 10: Tissue Doppler Measures of Diastole**




Em: peak early myocardial diastolic velocity; Am: peak late myocardial diastolic velocity.

**Cardiac Parasympathetic Activity and Diastolic Function**

The effects of cardiac parasympathetic activity on LV diastolic function are not very well understood. In particular, there is a paucity in the literature on the interaction between cardiac autonomics and echocardiographic parameters of diastole in healthy populations. There is however evidence suggesting that vagal outflow may impact cardiac relaxation by altering atrial contractility. Generally, studies have shown that an increase in cardiac vagal outflow may reduce atrial contractility, whereas an attenuation in cardiac vagal outflow may increase atrial contractility. A study in dogs by Williams et al. (1965)[65] demonsrated a 19% reduction in atrial contractile force following vagal nerve stimulation, which was also later shown by Steusse et al. (1979)[66]. Likewise, another dog study showed that direct vagal nerve stimulation resulted in an increase in early diastolic filling by 24%, which was accounted for by an increase in left atrial pressure[67]. In human studies, Finkelhor et al., (1995) showed that the age induced increase in transmitral A velocity was closely related to the reduction in HRV ($r=-0.54$; $p=0.002$)[68]. Similarly, cholinergic blockade via atropine was shown to decrease E velocity by 23%, increase A velocity by 103%, and consequently decrease the E:A ratio by 60% [69]. This is accordance with results from Stratton et al. (2003) who showed a reduction in early diastolic filling following parasympathetic withdrawal in humans[70]. A more recent study showed that healthy participants who had an E:A ratio <1 demonstrated significantly lower HF power compared to those with an E:A ratio >1[11]. This suggests that those individuals with reduced HRV may demonstrate a reduction in the E:A ratio due to elevated atrial contraction. Accordingly, these studies suggest that in healthy normal conditions, elevated cardiac vagal outflow may increase atrial pressure thus increasing early diastolic

velocity. In contrast, a reduction in cardiac vagal outflow increases atrial contractility, thus making the LV rely more on late diastole.

**Preload and Diastolic Function**

Doppler indices of LV diastolic function are substantially governed by factors that affect LV and left atrial pressures. As mentioned before, normal early diastolic function is driven by a pressure gradient that results in rapid and passive blood flow from the atria to the ventricle. Therefore, early and late diastolic velocities are influenced by factors that simultaneously affect LV and atrial pressures. Thus, interventions that increase left atrial pressure would subsequently increase early diastolic velocity, whereas a reduction in left atrial pressure would attenuate early diastolic velocity[10]. Preload is partly defined by the pressure in the LV at the end of diastole. An increase in preload suggests an increase in cardiac filling, which can be due to increased blood volume, increased atrial contractility and/or increased ventricular elasticity[71]. The most direct evidence demonstrating the effect of preload on diastolic function can be derived from models of hemodialysis. Assa et al. (2013) reported a significant reduction in transmitral E and TD Em velocities after reduced preload during a dialysis session[72]. Additional Doppler measures of diastole were also impaired during hemodialysis, such as prolongation of DT and IVRT[72]. These results are in agreement with past studies showing diastolic impairment in response to reduced preload[73,74]. A dose dependent reduction in transmitral E velocity has also been demonstrated in response to gradual increases in lower body negative pressures, whereas a dose dependent increase in transmitral E velocity has been shown in response to gradual increases in saline infusion[75]. The same investigators also demonstrated that TD Em velocities increase after rapid saline infusion and decrease after caval occlusion[75].

Furthermore, Mak et al. (2013) demonstrated a significant increase in transmitral E and TD Em velocities in healthy individuals after increasing preload via rapid saline infusion and a high salt diet[76].

**Physical Activity and Diastolic Function**

Most of the knowledge obtained on cardiac adaptations to physical inactivity is derived from studies of bed rest and spaceflight. Bed rest and spaceflight models are used as analogues to severe physical inactivity in order to understand the cardio-physiological adaptations to sedentary life. Interestingly, most studies show that following a given period of physical inactivity, LV systolic function is unaltered whereas diastolic impairments start to develop. For example, Atkov et al. (1987) indicated that the reduction in stroke volume in astronauts following 8 months of spaceflight was due to hemodynamic alterations, as cardiac contractility was unaltered[77]. Similar observations of preserved systolic function have also been reported from bed rest studies lasting more than a year[78]. Moreover, Levine et al. (1997) showed that 2 weeks of bed rest resulted in a 17% reduction in plasma volume and concomitant impairments in ventricular filling[79]. Furthermore, Perhonen et al. (2001) reported a 14% reduction in LV end diastolic volume at 2 weeks of bed rest, and an additional 8% reduction by 12 weeks, resulting in reduced diastolic filling[80]. This was attributed to the well-documented reduction in plasma volume following a period of physical inactivity[81]. Moreover, Carrick-Ranson et al. (2013) examined LV diastolic function following a 5-week period of bed rest in an exercising and non-exercising group. Results showed that only the non-exercising group exhibited a significant reduction in E (79±17 to 67±14 cm $s^{-1}$: p= =0.001) and Em (14±3 to 13±3 cm $s^{-1}$; p=0.02), as well as an increase in IVRT (93±24 to 110±24 msec; p=0.02).

Interestingly, all of these parameters returned to baseline values following saline infusion, suggesting that reduced diastolic function following a period of physical inactivity may be influenced by changes in loading conditions rather than intrinsic ventricular properties[82].

**Spinal cord injury and diastolic function**

Hemodynamic variables of LV systolic function, such as ejection fraction and stroke volume, are typically unaltered in individuals with SCI[7,8,56,83] suggesting preserved systolic function in this population. Findings on LV diastolic function in individuals with SCI remain equivocal however, as previous reports suggest preserved diastole while more recent studies demonstrate impaired diastolic function. For example, De Groot et al. (2006) reported similar diastolic function between individuals with complete tetraplegia and able-bodied individuals via pulse-wave Doppler, TD and pulmonary parameters of diastole[8]. Similar results of preserved diastolic function have also been reported in individuals with paraplegia[84], older sedentary individuals with tetraplegia[9], untrained individuals with SCI[85] and rugby athletes with SCI[86]. All of these studies also report an "adaptive" reduction in cardiac dimensions in response to the reduced venous return that is accompanied by SCI. It has been hypothesized that such a reduction in cardiac dimensions maintains the mass:volume ratio and preserves cardiac wall stress, which may explain the preserved diastolic performance in those with SCI[8,86].

However, more recent studies on diastolic function in SCI contradict the aforementioned findings. For example, Driussi et al. (2014) showed that individuals with complete paraplegia had significantly lower transmitral E velocity, E:A ratio as well as TD Em compared to able-bodied controls[6]. In addition, results from Matos-Souza et al.

(2010) showed that individuals with SCI had a lower Em compared to controls, as well as a higher LV filling pressure[7]. In addition, the participants who had an E:Em ratio greater than eight exhibited elevated relative wall thickness and lower peak myocardial systolic velocity, both of which are patterns associated with diastolic heart failure[10]. In order to determine the effects of physical activity on diastolic function in SCI, Rossi et al. (2014) compared echocardiographic diastolic parameters between physically active SCI (SCI-A), sedentary SCI (SCI-S) and able-bodied controls. Results showed that SCI-S presented higher E:Em ratio compared to SCI-A and controls (SCI-S: 8.0±0.5; SCI-A: 6.4±0.3; AB: 5.9±0.3; $p<0.05$) and lower Em:Am ratio (SCI-S: 1.18±0.09; SCI-A: 1.57±0.12; controls: 1.63±0.08; $p<0.05$)[83]. When comparing SCI groups according to level of injury, sedentary tetraplegics had significantly lower Em compared to active tetraplegics (8.6±0.5cm/s vs. 10.9±0.6cm/s; $p<0.05$). These results are in agreement with findings by Schreiber et al. (2014) who reported significantly lower E:A ratio in SCI-S compared to SCI-A (1.43±0.09 vs 1.71±0.08; $p=0.02$) as well as lower Em (9.3±0.6cm/s vs 11.0±0.6cm/s; $p=0.04$) and higher E:Em ratio (7.7±0.5 vs 6.4±0.3; $p=0.02$)[87].

It is important to note that Doppler indices of LV diastolic function are highly influenced by preload[88]. After SCI, there is a hemodynamic shift towards reduced preload as a result of reduced venous return. Such a reduction in venous return can be explained by the lower total blood volume seen in individuals with SCI[89], reduced vascular sympathetic tone below the injury[90] and ablation of the muscle pump effect. One or a combination of all these factors can collectively reduce the amount of blood that returns to the heart, thus decreasing cardiac filling. Accordingly, it is possible that the reported alterations in LV diastolic function after SCI are a result of reduced preload rather than

intrinsic architectural alterations in the LV. In addition, individuals with SCI may demonstrate impaired cardiac vagal outflow as a result of the profound physical inactivity which typically occurs after paralysis. As reported above, alterations in cardiac vagal activity can also have an influence on LV diastolic function. Therefore, it is possible that the reported alterations in LV diastole in those with SCI could be related to autonomic impairments. Accordingly, further studies are warranted in order to elucidate the mechanisms behind the reported diastolic impairments in individuals with SCI.

# References:


1. Zile MR, Brutsaert DL: New concepts in diastolic dysfunction and diastolic heart failure: part 1. Diagnosis, prognosis and measurements of diastolic function. Circulation. 2002, 105:1387-93.

2. Brubaker P, Kaminsky L, Whaley M: Coronary artery disease: essentials of prevention and rehabilitation programs. Muncie, Indiana: Human Kinetics. 2002, Coronary artery disease: essentials of prevention and rehabilitation programs. Muncie, Indiana: Human Kinetics

3. Billman GE: The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol 2013, 4.

4. Sharif H, Millar PJ, Incognito AV, Ditor DS. Non-invasive electrocardiographic assessments of cardiac autonomic modulation in individuals with spinal cord injury. Spinal cord 2016, 54:166-171.

5. Piccirillo G, Magri D, Ogawa M et al: Autonomic nervous system activity measured directly and qt interval variability in normal and pacing-induced tachycardia heart failure dogs. J A Coll Cardiol 2009, 54: 840-850.

6. Driussi C, Ius A, Bizzarini E, et al: Structural and function left ventricular impairment in subjects with chronic spinal cord injury and no overt cardiovascular disease. J Spinal Cord Med 2014, 37:85-92.

7. Matos-Souza JR, Pithon KR, Oliveira RT, et al: Altered left ventricular diastolic function in subjects with spinal cord injury. Spinal Cord 2010, 49:65-69.

8. De Groot PC, Van Dijk A, Dijk E, Hopman MT: Preserved cardiac function after chronic sci. Arch Phys Med Rehabil 2006, 87:1195-2000.

9. Eysmann SB, Douglas PS, Katz SE, Sarkarati M, Wei JY: Left ventricular mass and diastolic filling patterns in quadriplegia and implications for effects of normal aging on the heart. AM J Cardiol 1995, 75:201-203.

10. Gaasch W, Zile M: Left ventricular diastolic dysfunction and diastolic heart failure. Annu Rev Med 2004, 55:373-394.

11. Xenopoulos NP, Applegate RJ: The effect of vagal stimulation on left ventricular systolic and diastolic performance. Am J Physiol Heart Circ Physiol 1994, 266: H2167-H2173.

12. Stratton JR, Levy WC, Caldwell JH: Effects of aging on cardiovascular responses to parasympathetic withdrawal. J Am Coll Cardiol 2003, 41:2077-2083.

13. Antelmi I, Yamada AT, Hsin CN, TsuTsui JM, Grupi CJ, Mansur AJ: Influence of parasympathetic modulation in Doppler mitral inflow velocity in individuals without heart disease. J Am Soc Echocardiog 2010, 23:762-765.

14. Poirier P, Bogaty P, Philippon F, et al: Preclinical diabetic cardiomyopathy: relation of left ventricular diastolic dysfunction to cardiac autonomic neuropathy in men with uncomplicated well-controlled type 2 diabetes. Metabolism 2001, 52:1056-1061.

15. Krenz NK, Weaver LC: Changes in the morphology of sympathetic preganglionic neurons parallel the development of autonomic dysreflexia after spinal cord injury in rats. Neuroscience Letters 1998, 243:61-64.

16. Farry A, Baxter D: The incidence and prevalence of spinal cord injury in Canada: overview and estimates based on current evidence. Rick Hansen Institute. 2010.

17. Dryden DM, Saunders LD, Rowe BH, et al: The epidemiology of traumatic spinal cord injury in Alberta, Canada. Can J Neurol Sci 2003, 30:113:121.

18. Pirouzmand F: Epidemiological trends of spine and spinal cord injuries in the largest Canadian adult trauma center from 1986 to 2006. J Neurosurg Spine 2010, 12:131-140.

19. Furlan JC, Fehling MG: Cardiovascular complications after acute spinal cord injury: pathophysiology, diagnosis, and management. Neurosurg Focus 2008, 25:1-15.

20. Garstang SV, Miller-Smith SA: Autonomic nervous system dysfunction after spinal cord injury. Phys Med Rehabil Clin N Am 2007, 18:275-296.

21. Hockman CH: Transmission of impulses in the autonomic nervous system. In: Essentials of autonomic function. Charles C Thomas Publishing. Springfield, Illinois.1987, 23-32.

22. Lehmann KG, Lane JG, Piepmeier JM, Batsford WP: Cardiovascular abnormalities accompanying acute spinal cord injury in humans: incidence, time course and severity. J Am Coll Cardiol 1987, 10:46-52.

23. Claydon VE, Steeves JD, Krassioukov A: Orthostatic hypotension following spinal cord injury: understanding clinical pathophysiology. Int Spinal Cord Soc 2006, 44:341-351

24. Bycroft J, Shergill IS, Choong EA, Arya N, Shah PJ: Autonomic dysreflexia: a medical emergency. Postgrad Med J 2005, 81:232-235.

25. Myers J, Lee M, Kiratli WJ: Cardiovascular disease in spinal cord injury an overview of prevalence, risk, evaluation, and management. Am J Physica Med Rehabil 2007, 86:142-152.

26. Furlan JC, Fehlings MG, Shannon P, et al: Descending vasomotor pathways in humans: correlation between axonal preservation and cardiovascular dysfunction after spinal cord injury. J Neurotrauma 2003, 20:1351-1363.

27. Magnano AR, Holleran S, Ramakrishnan R, Raffiel JA, Bloomfield DM: Autonomic nervous system influence on QT interval in normal subjects. J AM Coll Cardiol 2002, 39:1820-1826.

28. Verrier RL, Antzelevitch C: Autonomic aspects of arrhythmogenesis: the enduring and the new. Curr Opin Cardiol 2004, 19:2-11.

29. Antzelevitch C, Fish J: Electrical heterogeneity within the ventricular wall. Basic Res Cardiol 2001, 96:517-527.

30. Askelrod SD, Gordon FA, Ubel DC, Shannon AC, Berger, Cohen, RJ: Power spectrum analysis of heart rate fluctuations: a quantitative probe of beat-to-beat cardiovascular control. Science 1981, 213:220-222.

31. Warner HR, Cox A: A mathematical model of heart rate control by sympathetic and vagus afferent information. J Appl Physiol 1962, 17:349-355.

32. Cam AJ, Malik M, Bigger JT, et al: Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 1996, 93:1043-1065.

33. Kammath MV, Fallen EL: Power spectral analysis of heart rate variability. A non-invasive signature of cardiac autonomic function. CRC Crit Rev Biomed Eng 1993. 21:245-311.

34. Pomeranze B, Macaulay RJ, Caudill MA, et al: Assessment of autonomic function in humans by heart rate spectral analysis. Am J Physiol. 248:H151-H153.

35. Cotie LM, St. Amand T, McMilan R, Picton P, Ditor DS: Effects of autonomic blockade on heart rate variability after spinal cord injury: A case series. Crit Rev Phys Rehabil Med 2010, 22:1-12.

36. Randall DC, Brown DR, Raisch RM, Yingling JD, Randall WC: SA nodal parasympathectomy delineates autonomic control of heart rate power spectrum. Am J Physiol Heart Circ Physiol 1991, 260:H985-H988.

37. Houle MS, Billman GE: Low-frequency component of the heart rate variability spectrum: a poor marker of sympathetic activity. Am J Physiol Heart Circ Physiol 1999, 276:H215-H223.

38. Piccirillo G, Ogawa M, Song J, et al: Power spectral analysis of heart rate variability and autonomic nervous system activity measured directly in healthy dogs and dogs with tachycardia-induced heart failure. Heart Rhythm 2009, 6:546-552.

39. Antzelevitch C, Fish J: Electrical heterogeneity within the ventricular wall. Bas Res Cardiol 2001, 96:517-527.

40. Yan GX, Antzelevitch C: Cellular basis for the normal T wave and the electrocardiographic manifestations of the long-QT syndrome. Circulation 1998, 98:1928-1936.

41. Berger RD, Kasper EK, Baughman KL et al: Beat-to-beat QT interval variability. Novel evidence for repolarization lability inischemic and nonischemic dilated cardiomyopathy. Circulation 1997, 96:1557-65.

42. Ahnve S, Vallin H: Influence of heart rate and inhibition of autonomic tone on the QT interval. Circulation 1989, 65:435-439.

43. Yeragani VK, Pohl R, Jampala VC, Balon R, Kay J, Igel G: Effect of posture and isoproterenol on beat-to-beat heart rate and qt variability. Neuropsychology 2000, 41:113-123.

44. Baumert M, Schlaich PM, Nalivaiko E, et al: Relation between QT interval variability and cardiac sympathetic activity in hypertension. Am J Physiol Heart Circ Physiol 2011, 300:H1412-H1417.

45. Piccirillo G, Magnanti M, Matera S, et al: Age and QT variability index during free breathing, controlled breathing and tilt in patients with chronic heart failure and healthy control subjects. Transl Res 2006, 148:72-78.

46. Yeragani VK, Pohl R, Jampala VC, et al: Increased QT variability in patients with panic disorder and depression. Psych Res 2000b, 93:225-235.

47. Piccirillo G, Cacciafesta M, Lionetti, et al: Influence of age, the autonomic nervous system and anxiety on QT-interval variability. Clinical Sci 2001, 101:429-438.

48. Khandoker AH, Imam MA, Couderc JP, Palaniswami M, Jelinek H: QT variability index changes with severity of cardiovascular autonomic neuropathy. IEEE Trans Inf Technol Biomed 2012, 16:900-906.

49. Ditor DS, Kamath MV, MacDonald MJ, et al. Reproducibility of heart rate variability and blood pressure variability in individuals with spinal cord injury. Clin Auton Res 2005, 15:387-393.

50. Inoue K, Miyake S, Kumashiro M, Ogata H, Ueta T, Akatsu T: Power spectral analysis of blood pressure variability in traumatic quadriplegic humans. Am J Physiol 1990, 260:H842-H847

51. Koh J, Brown TE, Beightol LA, Ha CY, Eckberg DL: Human autonomic rhythms: vagal cardiac mechanisms in tetraplegic subjects. J Phys 1994, 474:483-95.

52. Guzzetti S, Cogliati C, Broggi C, et al: Influences of neural mechanisms on heart period and arterial pressure variabilities in quadriplegic patients. Am J Physiol 1994, 35:H1112-H1120.

53. Inoue K, Ogata H, Hayano J: Assessment of autonomic function in traumatic quadriplegic and paraplegic patients by spectral analysis of heart rate variability. J Auton Nerv Sys 1995, 54:225-234.

54. Grimm DR, DeMeersman RE, Almenoff PL, Spungen AM, Bauman WA: Sympathovagal balance of the heart in subjects with spinal cord injury. Am J Physiol 1997, 272:H835-H842.

55. Wang YH, Huang TS, Lin JL, et al: Decreased autonomic nervous system activity as assessed by heart rate variability in patients with chronic tetraplegia. Arch Phys Med Rehabil 2000, 81:1181-1184.

56. Wecht JM, Weir JP, Bauman WA: Blunted heart rate response to vagal withdrawal in persons with tetraplegia. Clinical Autonomic Research 2006a, 16: 378-383.

57. Wecht JM, Weir JP, DeMeersman RE, et al: Cold face test in persons with spinal cord injury: age versus inactivity. Clin Auton Res 2009, 19:221-229.

58. Ditor DS, Kamath MV, MacDonald MJ, et al: The effects of body weight supported treadmill training on heart rate variability and blood pressure variability in individuals with spinal cord injury. J Appl Physiol 2000b, 98:1519-1525.

59. Zamunér AR, Silva E, Teodori RM, Catai AM, Moreno MA: Autonomic modulation of heart rate in paraplegic wheelchair basketball players: Linear and nonlinear analysis. J Sports Sci 2003, 31:396-404

60. Wecht JM, Marsico R, Weir JP, Spungen AM, Bauman WA, De Meersman RE: Autonomic recovery from peak arm exercise in fit and unfit individuals with paraplegia. Med SCI Sports Exerc 2006b, 38:1223-1228.

61. La Fountaine MF, Wecht JM, Rosado-Rivera D, Cirnigliaro CM, Spungen AM, Bauman WA: The QT variability index and cardiac autonomic modulation: perspectives from apparently healthy men with spinal cord injury. Cardiology 2010, 117:253-259.

62. Ravensbergen HJ, Walsh ML, Krassioukov AV, Claydon VE: Electrocardiogram-based predictors for arrhythmia after spinal cord injury. Clinic Auton Res 2012, 22:265-273.

63. Vendelin M, Bovendeerd PH, Engelbrecht J, Arts T: Optimizing ventricular fibers uniform strain or stress, but not ATP consumption, leads to high efficiency. Am J Physiol Heart Circ Physiol 2002, 283:H1072–H1081.

64. Sengupta P, Krishnamoorthy V, Korinek J, et al: Left ventricular form and function revisited: Applied translational science to cardiovascular ultrasound imaging. J Am Soc Echocardiog 2007, 20:539-551.

65. Williams JF, Sonnenblick EH, Braunwald E: Determinants of atrial contractile force in the intact heart. Am J Physiol Leg Cont 1965, 209:1061-1068.

66. Stuesse SL, Wallick DW, Levy MN: Autonomic control of right atrial contractile strength in the dog. Am J Physiol Heart Circ Physiol 1979, 236:H860-H865.

67. Xenopoulos NP, Applegate RJ: The effect of vagal stimulation on left ventricular systolic and diastolic performance. Am J Physiol Heart Circ Physiol 1994, 266:H2167-H2173.

68. Finkelhor RS, Karwa P, Kaurman E, Bahler RC: The relationship of autonomic function to age-related changes in left ventricular filling in normal subjects. Euro Heart J 1995, 16: 1716-1720.

69. Johannessen KA, Cerqueira M, Veith RC, Stratton JR: Influence of sympathetic stimulation and parasympathetic withdrawal on Doppler echocardiographic left ventricular diastolic filling velocities in young normal subjects. Am J Cardiol 1991, 67:520-526.

70. Stratton JR, Levy WC, Caldwell JH: Effects of aging on cardiovascular responses to parasympathetic withdrawal. J Am Coll Cardiol 2003, 41:2077-2083.

71. Klabunde R: *Cardiovascular Physiology Concepts. Second Edition.* Baltimore, MD: Lippincott Williams & Wilkins, a Wolters Kluwer business 2012.

72. Assa S, Hummel Y, Voors A: Changes in left ventricular diastolic function during hemodialysis sessions. Am J Kidney Dis 2013, 62:549-556.

73. Agmon Y, Oh J, McCarthy J, et al: Effect of volume reduction on mitral annular diastolic velocities in hemodialysis patients. Am J Cardiol 2000, 85:665-668.

74. Chakko S, Girgis I, Conteras G, et al: Effects of hemodialysis on left ventricular function evaluated by tissue Doppler imaging. Biomed Pharmacother 1997, 60:66-70.

75. Firstenberg MS, Greenberg NL, Main ML, et al. Determinants of diastolic myocardial tissue Doppler velocities: influences of relaxation and preload. Journal of Applied Physiology 2001, 90:299-307.

76. Mak GS, Sawaya H, Khan AM, et al: Effects of subacute dietary salt intake and acute volume expansion on diastolic function in young normotensive individuals. Eur Heart J Cardiovasc Imag 2013, 14:1092-1098.

77. Atkov O, Bednenko VS, Fomina GA: Ultrasound techniques in space medicine. Aviat Space Environ Med 1987, 58:A69-A73.

78. Turbasoc VD, Artamonova NP, Golubchikova ZA, Saikina GP, Nechavena EL, Antoneko LV: Cardiac bioelectrical activity in healthy men during 370-day head-down tilt hypokinesia. Aviakosm Ekolog Med 1992, 26:39-41.

79. Levine BD, Zuckerman JH, Pawelczyk JA: Cardiac atrophy after bed-rest deconditioning. A nonneural mechanism for orthostatic intolerance. Circulation 1997, 96:517-525.

80. Perhonen MA, Franco F, Lane LD, et al: Cardiac atrophy after bed rest and spaceflight. J App Physiol 2001, 91:645-53.

81. Leach CS, ALfrey CP, Suki WA, et al: Regulation of body fluid compartments during short-term spaceflight. J Appl Physiol 1996, 81:105-116.

82. Carrick-Ranson G, Hastings JL, Bhella PS, Shibata S, Levine BD: The effect of exercise training on left ventricular and diastolic suction at rest and during orthostatic stress after bed rest. Exp Physiol 2013, 98:501-513.

83. Rossi G, Matos-Souza J, Silva AC, et al: Physical activity and improved diastolic function in spinal cord injured subjects. Med Sci Spor Exerc 2014, 46:887-892.

84. Kessler KM, Pina I, Green B, et al: Cardiovascular findings in quadriplegic and paraplegic patients and in normal subjects. Am J Cardiol 1986, 58:525-530.

85. Maggioni MA, Ferratini M, Pezzano A, et al: Heart adaptations to long-term aerobic training in paraplegic subjects: an echocardiographic study. Spinal cord 2012, 50:538-542.

86. West CR, Campbell IG, Shave RE, Romer LM: Resting cardiopulmonary function in paralympic athletes with cervical spinal cord injury. Med Sci Sports Exerc 2012, 44:323-329.

87. Schreiber R, Paim LR, Rossi G, et al: Matrix metalloproteinases and left ventricular function and structure in spinal cord injured subjects. Clinica Chimica Acta 2014, 437:136-140.

88. Choong CY, Herrmann HC, Weyman AE, Fifer MA: Preload dependence of Doppler-derived indexes of left ventricular diastolic function in humans. J Am Coll Cardiol 1987, 10:800-808.

89. Houtman S, Berend O, Hopman M: Blood volume and hemoglobin after spinal cord injury. Am J Phys Med Rehabil 2000, 79:260-265.

90. Krassioukov A: Autonomic function following cervical spinal cord injury. Respir Physiol Neurobio 2009, 169:157-164.

## Chapter 3 – Purpose & Hypotheses

***Manuscript 1:*** **The influence of cardiac autonomic activity on the QT-variability index in able-bodied and incomplete spinal cord injured individuals.**

The purpose of this study was to use pharmacological blockade of the sympathetic and parasympathetic regulation of the heart to determine the relationship between the QTVI and cardiac autonomic regulation in individuals with autonomically incomplete SCI and able-bodied controls. We hypothesized that 1) the QTVI would increase with the transition from supine rest to cardiovascular stress, in both individuals with autonomic incomplete SCI and the able-bodied. Further, during cardiovascular stress, the QTVI will be significantly reduced with β-blockade in both groups. These findings would provide evidence that the QTVI is an index of cardiac sympathetic regulation during autonomic arousal. 2) we also hypothesized that during resting conditions, the QTVI will increase after parasympathetic blockade via atropine in both the autonomic incomplete SCI and able-bodied groups.

***Manuscript 2:*** **Reproducibility of the QT-variability index in individuals with spinal cord injury.**

The purpose of this study was to examine the day-to-day reproducibility of the QTVI in individuals with SCI. This was done via an intraclass correlation coefficiency test. We hypothesized that the QTVI would show good intraclacc correlation coefficiency values and high reproducibility as a measure of cardiac autonomic function.

***Manuscript 3:*** **Cardiac autonomic and ventricular diastolic interaction in individuals with spinal cord injury.**

This is an exploratory study aiming to examine 1) the relationship between resting cardiac parasympathetic activity and LV diastolic function in able-bodied and SCI individuals at rest and 2) LV diastolic changes in response to cardiac parasympathetic

stimulation via the cold face test (CFT) in able-bodied and SCI individuals. We hypothesized that 1) both able-bodied and SCI individuals would show positive correlations between cardiac parasympathetic activity and early diastolic filling velocity. 2) We also hypothesized that an increase in cardiac vagal outflow via the CFT would result in increased diastolic filling velocity in both groups.

***Manuscript 4:*** **The effect of blood volume and volume loading on left ventricular diastolic function in individuals with spinal cord injury.**

The purpose of this study was to determine if individuals with SCI demonstrate diastolic impairments at rest and/or during a volume stress induced by rapid saline infusion. We also aimed to determine if reductions in blood volume following SCI contribute to the putative diastolic impairments in this population. We hypothesized that individuals with SCI would demonstrate reduced diastolic function as a result of being hypovolemic. We also hypothesized that individuals with SCI would show normal diastolic responses to the rapid saline infusion.

**Chapter 4**
**Manuscript 1**

# The Influence of Cardiac Autonomic Activity on the QT-Variability Index in Able-Bodied and Incomplete Spinal Cord Injured Individuals

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## Abstract:

**Objectives:** To investigate, via autonomic blockade, if the QT-variability index (QTVI) is a measure of cardiac autonomic regulation in able-bodied (AB) and incomplete spinal cord injured (SCI) individuals.

**Methods:** Four SCI (41.6±13.4 years; C4-C7, AIS B-D, 13.4±13.4 years post injury) and 4 AB (33.0±7.8 years) individuals were tested. QTVI was determined from electrocardiographic readings obtained during supine rest and cardiovascular (CV) stress, with and without autonomic blockade. CV stress was induced by 40° head-up tilt, the hand submerged in 10°C water and the jaw clenched. Autonomic blockade was achieved with metoprolol (β-blockade) and atropine (cholinergic blockade).

**Results:** There was no group x condition interaction for QTVI, although there was a significant main effect for condition. After collapsing across groups, QTVI increased with CV stress ($p=0.01$) and decreased with subsequent β-blockade ($p=0.04$), suggesting that during CV stress, QTVI is reflective of cardiac sympathetic activity. During supine rest, β-blockade did not change QTVI ($p=0.24$), however, cholinergic blockade increased QTVI ($p<0.001$), suggesting that during rest, QTVI is inversely related to cardiac parasympathetic regulation.

**Conclusion:** During times of CV stress, QTVI reflects cardiac sympathetic activity, while during resting conditions, QTVI is inversely related to cardiac parasympathetic activity. These relationships persist after autonomic incomplete SCI.


**Key Words:** QTVI, Autonomic blockade, Spinal cord injury

## Introduction:

The QT interval on the electrocardiogram (ECG) corresponds to the duration of global ventricular repolarization. When analyzed on a beat-to-beat basis, the duration of a series of QT intervals shows a certain amount of variability, which can be quantified by the QT variability index (QTVI)[1]. From a clinical perspective, a rise in the QTVI is indicative of increased beat-to-beat repolarization variability, which is predictive of future arrhythmias and sudden cardiac death[2]. From a physiological perspective, previous literature has shown that QTVI may be influenced by the autonomic nervous system; specifically, sympathetic outflow to the heart in conditions of cardiac-related morbidity. This notion of sympathetic involvement is supported by clinical studies that examined disorders associated with elevated sympathetic tone, and experimental studies that induced sympathetic activation via head-up tilt or administration of sympathomimetic agents. Regarding the clinical studies, an elevated QTVI has been reported in individuals with cardiac conditions such as dilated cardiomyopathy[1], hypertension[3,4] , congestive heart failure[5] and chronic heart failure[6], all of which are associated with increased efferent sympathetic tone acting on the end-organ. An elevated QTVI has also been reported in non-cardiac conditions associated with elevated sympathetic activity, such as obstructive sleep apnea[7], panic disorder and depression[8]. Regarding the experimental studies, a head-up tilt test and the intravenous infusion of the β-adrenoceptor agonist isoprotenerol have been shown to increase QTVI in healthy individuals further demonstrating the sensitivity of QTVI to the physiological state[9]. However, other studies in animals[10] and humans[11] have provided evidence that the QTVI may be influenced by

vagal regulation as well. Piccirillo et al. (2009) demonstrated in animal models, that baseline resting QTVI was *inversely* correlated with vagal nerve activity[10].

Spinal cord injury (SCI) is associated with severe cardiac autonomic dysfunctions, mainly due to loss of supraspinal control over sympathetic regulation to the heart and vasculature[12]. Consequences of this impaired sympathetic regulation include bradycardia, due to unopposed vagal outflow to the sinoatrial node, and hypotension due to a loss of sympathetic outflow to the sublesional vessels[13]. Further, and unique to SCI, individuals with high paraplegia or tetraplegia may experience autonomic dysreflexia; a condition characterized by episodes of uncontrolled sympathetic reflexes that lead to elevations in blood pressure, which in severe cases, have caused cerebral hemorrhages or even death[14]. Despite the noted impairments in cardiac autonomic regulation after SCI, a consensus method of determining the amount of preserved cardiac autonomic function has yet to be developed for this population. Accordingly, the QTVI may potentially serve as a non-invasive method for measuring cardiac autonomic function in individuals with SCI, and previous work in this area has shown some promise. For example, La Fountaine et al. (2011) reported that individuals with tetraplegia, who have impaired cardiac autonomic regulation, exhibit higher QTVI values compared to healthy controls[11]. In addition, Ravensbergen et al. (2012) demonstrated higher QTVI values in those with autonomically complete SCI compared to those with autonomically incomplete SCI[15]. Therefore, it is clear that the QTVI is influenced by cardiac autonomic alterations after SCI, and it may have some diagnostic value. If the QTVI is a valid index of cardiac autonomic control, it may be of great use to the SCI population as a means to gauge deficits in cardiac autonomic regulation, and potential improvements with time, or

various forms of intervention (exercise, pharmacological, or neurological or otherwise). Accordingly, the purpose of this study was to use pharmacological blockade of the sympathetic and parasympathetic regulation of the heart to determine the relationship between the QTVI and cardiac autonomic regulation in individuals with autonomically incomplete SCI and able-bodied (AB) controls.

## Hypotheses:

We hypothesized that the QTVI is a useful surrogate of autonomic control in individuals with autonomic incomplete SCI, as it will reflect cardiac sympathetic regulation during autonomic arousal, and reflect cardiac parasympathetic regulation at rest. Thus, with respect to the current protocol, we hypothesized that:

1) the QTVI will increase with the transition from supine rest to cardiovascular stress, in both individuals with autonomic incomplete SCI and the able-bodied. Further, during cardiovascular stress, the QTVI will be significantly reduced with β-blockade in both groups. These findings would provide evidence that the QTVI is an index of cardiac sympathetic regulation during autonomic arousal.
2) during resting conditions, the QTVI will increase with decreases in cardiac parasympathetic regulation, in both individuals with autonomic incomplete SCI and the able-bodied. In other words, we hypothesized that during supine rest, the QTVI will increase with a decrease in parasympathetic regulation induced by muscarinic cholinergic blockade. These findings would provide evidence that the QTVI is inversely related to cardiac parasympathetic regulation during times of rest.

## Methods:

*Participants:*

Participants in the current study included 4 individuals with incomplete SCI (C4-C7, AIS B-D, 13.4±13.4 years post-injury) and 4 able-bodied individuals, matched for age, weight and height. No participant had any history of cardiovascular disease (CVD), as the QTVI is highly influenced by CVD. Participants were also required to be free of asthma and/or glaucoma, as these conditions are contraindications for metoprolol tartrate and atropine sulfate, respectively, which were used for the pharmacological blockade in this study. Likewise, participants were screened to exclude those who were taking any other medication that would contraindicate atropine sulfate and metoprolol tartrate administration. Screening prior to admission into this study, for both incomplete SCI and AB participants, was conducted by a physician and all participants provided informed consent prior to enrolling into this study. All participants gave their informed consent to participate in the study, and all procedures were approved by the Brock University Research Ethics Board and the Hotel Dieu Shaver Research Ethics Board.

*Study Design:*

The current investigation was a further analysis of data collected previously for the current cross-sectional investigation measured QTVI before and after pharmacological autonomic blockade in the supine and cardiovascular stress positions. Cardiovascular stress was induced by 40° head-up tilt, a submaximal isometric jaw contraction, and the right hand submerged in 10°C water. The water bath was maintained at 10°C throughout the entire protocol via a Polyscience 5000 Series chiller (Model 5205). These three stimuli were used in concert to ensure that the sympathetic nervous system was maximally activated. Previous research has shown that only when these three

manoeuvres were used together did sympathovagal balance increase significantly compared to supine baseline values in individuals with tetraplegia[16]. Further, Houtman et al., (2000), determined that head-up tilt alone was not sufficient to increase sympathetic activity in individuals with tetraplegia[17]. Autonomic blockade was performed by a Physician, and included the intravenous administration of metoprolol tartrate (Sabex Inc.) at a maximum dosage of 15mg (3x5mg), and atropine sulfate (Alveda Pharmaceuticals Inc.) at a dosage of 0.02 mg/kg. Atropine sulfate is a muscarinic cholinergic antagonist which blocks parasympathetic transmission to the sinoatrial node, while metoprolol tartrate is a $\beta_1$-adrenergic antagonist that blocks sympathetic transmission to the sinoatrial node. For the sake of safety, metoprolol tartrate administration was terminated once the heart rate (HR) reached a nadir, or if it was anticipated that the next 5mg dose would decrease the HR to less than 40 beats/min.

*Study Protocol:*

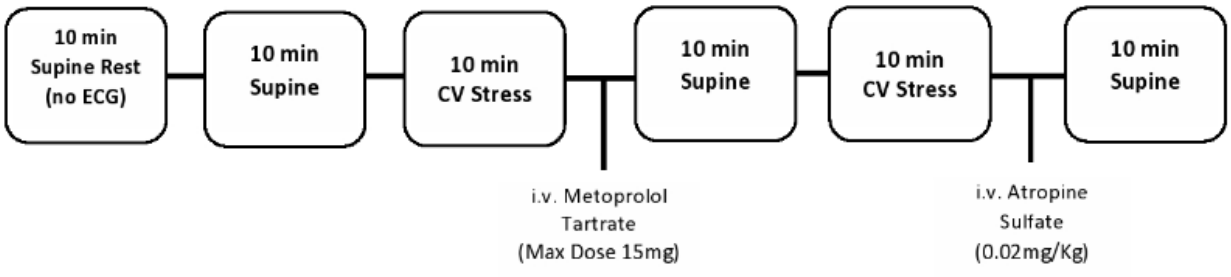
All participants were tested on two separate occasions. Visit 1 included a meeting with the Physician for medical clearance to participate in the study. This included screening for any history of cardiovascular disease, asthma, glaucoma, or smoking, as well as recording the cause of injury, and the level and severity of injury as determined by the American Spinal Injury Association Impairment Score (AIS). Participants were also familiarized with the study protocol and the laboratory setting, and informed consent was obtained.

During visit 2, participants reported to the laboratory for testing (Brock Heart Institute, St. Catharines, Canada). After voiding their bladders (to reduce the risk of autonomic dysreflexia during testing), participants were transferred to a tilt table, such

that no physical exertion was required by the participant. While supine, a catheter was inserted into the antecubital vein for drug administration, and the skin was prepared for electrode placement. Two separate electrocardiogram (ECG) recordings were taken during the testing protocol. For the sake of safety, participants had a 12-lead ECG to monitor for cardiac rhythm disturbances that could have occurred during the testing protocol, and a single-lead ECG to collect the continuous HR data for QTVI analysis using Power Lab and the software program Lab Chart 7 (ADInstruments). Both ECG recordings were collected at a sampling frequency of 1000Hz. To ensure a true resting baseline, all participants were required to lie supine on the tilt table in a dark quiet room for 10 minutes prior to data collection. Following this rest period, 10 minutes of baseline ECG was recorded in the supine position followed by another 10 minutes of ECG recording with the participant in the cardiovascular stress position. Following the cardiovascular stress position, participants were returned to the supine position. Once cardiovascular variables (blood pressure and HR) had returned to baseline, a maximum dose of 15mg of metoprolol tartrate was administered intravenously in three 5mg doses. Each 5mg dose was given over a 5 minute period with 5 minutes of rest between doses. As mentioned, metoprolol tartrate administration was terminated once the HR reached a nadir or if it was anticipated that another dose would decrease HR to less than 40 beats/min. Following this administration, ECG was again recorded for 10 minutes in the supine position and then for 10 minutes in the cardiovascular stress position. Participants were then returned to the supine position again and given intravenous administration of atropine sulfate at a dosage of 0.02 mg/kg[16]. Again, ECG was recorded for 10 minutes in the supine position. This entire protocol is represented in Figure 1. Participants refrained

from smoking, alcohol or caffeine consumption within twelve hours of the second laboratory visit. Likewise, medications were not interrupted during the study however, only baclofen and ditropan may have affected our measures. Although all of the incomplete SCI participants were taking these medications, the cardiovascular side effects are rare and transient[18].

**Figure 1: Study Protocol**




CV: cardiovascular stress, which involved 40° head-up tilt, a submaximal isometric jaw contraction and the right hand submerged in 10°C water.

*QT Interval and Variability Analysis:*

Electrocardiographic (ECG) signals for the current study were a further analysis of previously collected data by our group, for the assessment of heart rate variability (HRV) under different conditions[19]. None of the participants showed any arrhythmia and less that 1% of the beats were ectopic. For the purpose of the current study, ECG analysis was performed on 256 stable, artifact-free beats from the 10 minute continuous ECG recording in each experimental condition. A 50Hz low-pass filter was implemented to eliminate high frequency (HF) noise in order to obtain the clearest possible ECG signals for the analysis. The QT interval was determined as the time duration between the onset of the Q-wave, and the intersection point of the isoelectric line with the T-end. The T-end

was determined by creating a tangent line, fitted by least squares, to the descending slope of the T-wave over a data range 70%-30% of T-peak. Prior to further analysis, each beat was visually examined in order to ensure that the software determined and marked the QT intervals appropriately. The QTVI was calculated using the following formula developed by Berger et al., (1997):

$$QTVI = Log_{10} [(QTv/QTm^2) / (RRv/RRm^2)]$$

where, QTv is the QT interval variance, $QTm^2$ is the mean QT interval squared, RRv is the RR interval variance and $RRm^2$ is the mean RR interval squared[1] .

The QT interval corrected for heart rate (QTc) was also calculated by using the subject specific method developed my Malik et al., since traditional formulas of calculating QTc can produce misleading results when used in conjunction with HR altering drugs[20].

*Statistical Analysis:*

Two-way (group x condition) repeated measure of analyses of variance (ANOVA) were used to determine differences in QTVI, QTv, and RRv. Bonferroni's post-hoc test was used to compare means if significant interactions or main effects were found. One-way repeated measures ANOVA were used to determine differences in HR, QT, QTc and RRv between conditions and the Bonferroni's post-hoc test was used to compare means when significant main effects were found. A student's *t* test was used to determine differences in QTVI between groups during supine rest and to determine differences in mean arterial pressure (MAP) between conditions and anthropometric measurements between groups. All statistical analyses were performed using SPSS

software, all values were reported as means ± standard deviation, and the level of statistical significance was set at $p < 0.05$.

## Results:

Participant characteristics are shown in Table 1. There were no significant differences between groups for age, weight or height. However, the SCI group consisted of 4 males where AB group consisted of 3 males and 1 female. In addition, the SCI group did not show significant changes in MAP when moved from the supine condition to the CV stress condition (87.0 ± 8.7 mmHg to 81.7 ± 8.5 mmHg; p=0.11), and its HR responses to autonomic stimuli were similar to that exhibited by the AB group (Table 3), suggesting that this cohort of SCI individuals is autonomically incomplete.

Table 1: Participant characteristics

| **Characteristics** | **Able-Bodied** | **SCI** | ***p* value** |
|---|---|---|---|
| Age | 33.0 ± 7.8 | 41.6 ± 13.4 | 0.37 |
| Sex | 3 Male, 1 Female | 4 Male | N/A |
| Weight (Kg) | 83.0 ± 18.9 | 70.8 ± 15.2 | 0.27 |
| Height (cm) | 176.3 ± 9.8 | 174.7 ± 10.8 | 0.50 |
| Years Post Injury | NA | 13.4 ± 13.4 | |
| Level of Injury | NA | 1 C4; 3 C5 | |
| AIS Score | NA | 2 B; 2 C | |

AIS: AISA Impairment Score, where ASIA denotes American Spinal Injury Association

*QTVI:*

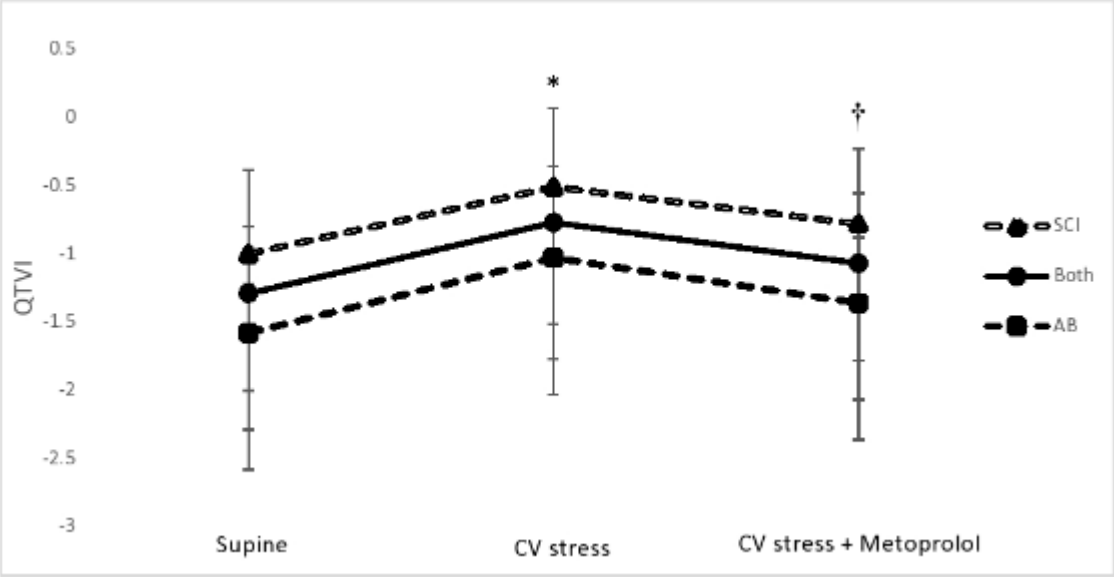
There was no significant difference between groups for QTVI during supine resting conditions (AB: -1.6 ± 0.4; SCI: -1.0 ± 0.6; p= 0.20).

*Regulation of cardiac sympathetic outflow and QTVI:*

The results of the 2-way ANOVA revealed no significant group x condition interaction for QTVI (p=0.97) nor a significant main effect for group (p=0.14). However, there was a significant main effect for condition (p=0.003). Post-hoc analysis of this main effect showed a significant increase in QTVI when participants (all participants, collapsed across group) were moved from the supine to the CV stress position (p=0.01). Post hoc analysis also showed that when participants (all participants, collapsed across group) were in the CV stress position (autonomically aroused) the QTVI was significantly reduced by the administration of metoprolol (p=0.04) (Figure 2). QT-variability index data for each participant during sympathetic regulation is provided in Table 2. Changes in the QTVI can be caused by either changes in the QTv or the RRv. As such, we analyzed these variables as well to determine which was responsible for the observed changes in QTVI caused by CV stress and β-blockade. Two-way ANOVA showed that there was no significant group x condition interaction for QTv (p=0.62), nor a significant main effect for group (p=0.36), however, there was a significant main effect for condition (p=0.02). Post hoc analysis showed a significant increase in QTv when participants were moved from the supine to the CV stress position (p=0.02), but unlike QTVI, QTv did not change after metoprolol administration (p=0.25) (Table 3). Two-way ANOVA also showed that there was no significant group x condition interaction for RRv (p=0.21), and no main effect for either group (p=0.13) or condition (0.47) (Table 3).

These results show that QTv behaves in a similar manner to QTVI in response to autonomic stimulation. This suggests that sympathetically-induced elevations in QTVI may be due to changes in QTv and not RRv.

**Figure 2: QTVI and Cardiac Sympathetic Regulation**




There was no significant group x condition interaction, and therefore the data is presented for each group (dotted lines) and collapsed across groups (solid line). Significant changes pertain to the collapsed data. Values are means ± standard deviation.

* denotes a significant increase (p=0.01) in QTVI compared to the supine condition (collapsed across groups)

† denotes a significant decrease (p=0.04) in QTVI compared to the CV stress condition (collapsed across groups).

**Table 2. Cardiac Sympathetic Regulation and Individual QTVI Values**

| Group | Participant | Supine | CV Stress | CV Stress + Met |
|---|---|---|---|---|
| **SCI** | 1 | -0.3 | 0.3 | -0.01 |
| | 2 | -1.1 | -1.0 | -1.3 |
| | 3 | -1.8 | -0.9 | -0.9 |
| | 4 | -0.9 | -0.4 | -0.9 |
| **AB** | 1 | -1.1 | -0.7 | -1.0 |
| | 2 | -1.9 | -1.3 | -1.9 |
| | 3 | -1.8 | -1.1 | -0.9 |
| | 4 | -1.5 | -1.1 | -1.5 |

SCI: Spinal cord injury; AB: Able-bodied; CV: Cardiovascular; Met: Metoprolol

**Table 3**: **Cardiac Sympathetic Regulation and QTv, RRv and HR**

| | Group | Condition | | |
|---|---|---|---|---|
| | | **Supine** | **CV stress** | **CV stress + Metoprolol** |
| **QTv (ms)** | **SCI** | 44.9 ± 61.9 | 73.0 ± 80.1 | 38.5 ± 38.4 |
| | **AB** | 8.4 ± 2.8 | 38.7 ± 17.1 | 19.9 ± 15.8 |
| | **Collapsed** | 26.7 ± 32.4 | 55.8 ± 48.6* | 29.2 ± 27.1 |
| **RRv (ms)** | **SCI** | 2705.7 ± 2036.8 | 1326.5 ± 916.8 | 1355.4 ± 703.9 |
| | **AB** | 3233.7 ± 1770.0 | 3240.7 ± 1348.8 | 3881.4 ± 2276.4 |
| | **Collapsed** | 2969.7 ± 1903.5 | 2283.6 ± 1132.9 | 2618.4 ± 1490.2 |
| **HR (beats/min)** | **SCI** | 57.9 ± 6.5 | 67.9 ± 11.6 | 64.7 ± 12.3 |
| | **AB** | 51.5 ± 3.8 | 59.0 ± 6.3 | 51.2 ± 3.6 |
| | **Collapsed** | 54.7 ± 5.2 | 65.7 ± 9.9 | 58.0 ± 8.0 |

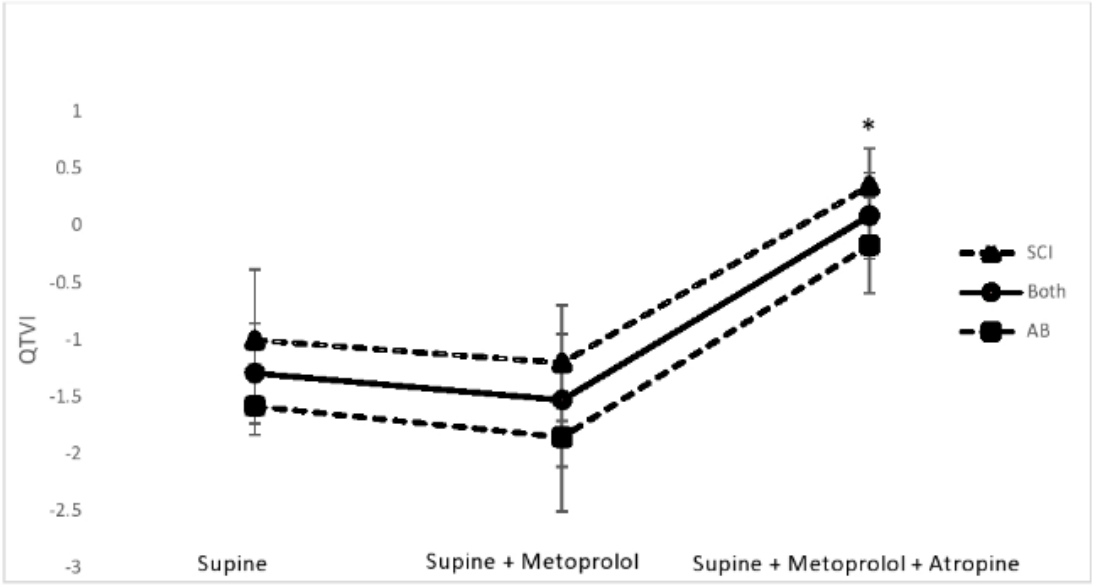
AB: Able-bodied; CV: Cardiovascular; SCI: Spinal cord injury; QTv: QT-interval variance; RRv: RR-interval variance; HR: Heart rate; *denotes significantly different from Supine ($p<0.05$)

*Regulation of cardiac vagal outflow and QTVI:*

The results of the 2-way (group x condition) ANOVA showed no significant interaction for QTVI ($p=0.89$) nor a significant main effect for group ($p=0.12$). However, there was a significant main effect for condition ($p<0.001$). Post-hoc analysis of this main effect (for all participants, collapsed across group) showed that the administration of metoprolol during the supine position caused no significant changes in QTVI ($p=0.24$). However, the administration of atropine caused a significant increase in QTVI compared to the other two conditions; the supine condition ($p<0.001$) and the supine condition with metoprolol ($p<0.001$) (Figure 3). QT-variability index data for each participant during parasympathetic regulation is provided in Table 4. Again, changes in the QTVI can be caused by either changes in the QTv or the RRv. As such, we analyzed these variables as well to determine which was responsible for the observed changes in QTVI caused by cholinergic blockade. The results of the 2-way ANOVA showed no group x condition interaction for QTv ($p=0.62$), nor a significant main effect for group ($p=0.30$) or condition ($p=0.22$) (Table 5). For RRv, the results of the 2-way ANOVA showed that there was no group x condition interaction ($p=0.24$) nor a significant main effect for group ($p=0.34$). There was, however, a significant main effect for condition ($p<0.001$) (Table 5). Post-hoc analysis of this main effect showed that when participants were in the supine position, the administration of metoprolol resulted in a trend towards an increase in RRv ($p=0.09$). Post-hoc analysis also showed that atropine administration caused a significant decrease in RRv compared to the other two conditions; the supine condition with no drug administration ($p=0.02$) and the supine condition with metoprolol ($p=0.01$) (Table 5). These results suggest that RRv responds to vagal regulation during resting

supine conditions, and suggest that changes in QTVI during resting supine conditions may be due to changes in RRv and not QTv.

**Figure 3: QTVI and Cardiac Parasympathetic Regulation**




There was no significant group x condition interaction, and therefore the data is presented for each group (dotted lines) and collapsed across groups (solid line). Significant changes pertain to the collapsed data. . Values are means ± standard deviation.

* denotes a significant increase ($p<0.001$) in QTVI compared to the supine condition (collapsed across groups) and the supine + metoprolol condition (collapsed across groups) .

**Table 4: Cardiac Parasympathetic Regulation and Individual QTVI Values**

| Group | Participant | Supine | Supine + Met | Supine + Met + Atrop |
|---|---|---|---|---|
| **SCI** | 1 | -0.3 | -0.5 | 0.4 |
| | 2 | -1.1 | -1.4 | 0.8 |
| | 3 | -1.8 | -1.7 | 0.02 |
| | 4 | -0.9 | -1.2 | 0.2 |
| **AB** | 1 | -1.1 | -1.3 | 0.4 |
| | 2 | -1.9 | -2.5 | -0.6 |
| | 3 | -1.8 | -2.3 | -0.3 |
| | 4 | -1.5 | -1.3 | -0.2 |

AB: Able-bodied; Atrop: Atropine CV: Cardiovascular; Met: Metoprolol; SCI: Spinal cord injury

**Table 5: Cardiac Vagal Regulation and QTv, RRv and HR**

| | Group | Condition | | |
|---|---|---|---|---|
| | | **Supine** | **Supine + Metoprolol** | **Supine + Metoprolol + Atropine** |
| **QTv (ms)** | **SCI** | 44.9 ± 61.9 | 31.5 ± 28.9 | 47.2 ± 39.1 |
| | **AB** | 8.43 ± 2.7 | 9.4 ± 5.1 | 28.6 ± 35.5 |
| | **Collapsed** | 26.7 ± 32.4 | 20.5 ± 17.0 | 38.0 ± 37.3 |
| **RRv (ms)** | **SCI** | 2705.6 ± 2036.8 | 3658.9 ± 1485.3 | 121.2 ± 94.9 |
| | **AB** | 3233.7 ± 1770.0 | 6624.8 ± 4243.1 | 129.1 ± 25.5 |
| | **Collapsed** | 2969.7 ± 1903.5 | 5141.9 ± 2864.2 | 125.2 ± 60.2*† |
| **HR (beats /min)** | **SCI** | 57.9 ± 6.5 | 57.9 ± 6.4 | 83.0 ± 15.3 |
| | **AB** | 51.5 ± 3.8 | 44.8 ± 2.5 | 83.2 ± 29.0 |
| | **Collapsed** | 54.7 ± 5.2 | 51.4 ± 4.5 | 83.2 ± 22.2 |

AB: Able-bodied; CV: Cardiovascular; SCI: Spinal cord injury
*denotes significantly different from Supine ($p<0.05$)
† denotes significantly different from Supine + Metoprolol ($p<0.05$)

**Discussion:**

The main findings of the current investigation were that in both AB participants and those with autonomically incomplete SCI, 1) QTVI significantly increased at times of elevated CV stress, 2) administration of the β-adrenoceptor antagonist, metoprolol, during CV stress significantly reduced QTVI and 3) in supine resting conditions, the administration of atropine significantly increased QTVI. These findings demonstrate that the QTVI is indeed influenced by cardiac autonomic activity, both sympathetic and parasympathetic, depending on the dominant system. The increase in QTVI during CV stress and its subsequent reduction after administration of metoprolol suggest that QTVI may reflect sympathetic activity at times of elevated CV stress. However, the rise in QTVI in response to atropine administration during supine resting conditions suggests that it may be inversely related to vagal activity during rest.

According to previous literature, QTVI is thought to be a reflection of sympathetic outflow to the heart. Yeragani et al. (2000b) demonstrated, in healthy adults, an increase in QTVI in response to isoprotenerol infusion and after head-up tilt, both of which are associated with increased sympathetic tone[9]. Also, administration of the β-blocker propranolol resulted in the attenuation of QT variability during atrial pacing in individuals without structural heart disease[21]. In addition, individuals with chronic cardiovascular morbidity exhibit higher QTVI values compared to healthy controls[3-7]. Although chronic cardiac conditions are associated with autonomic imbalances, they are also characterized by end organ pathology that may contribute to the elevated repolarization lability. Pathological alterations to the myocardium result in beat-to-beat action potential duration variability, rendering the myocardium unstable and predisposing

it to arrhythmias[22]. Prolongation of the QT interval is also seen in cardiomyopathies, which predisposes the myocardium to early after depolarization and may increase transmural dispersion of repolarization, both of which increase the risk of arrhythmias[23,24]. Therefore, individuals with cardiomyopathies likely demonstrate an increase in QTVI due to a combination of myocardial damage and elevations in sympathetic tone.

Spinal cord injury on the other hand, is not necessarily associated with myocardial damage. Accordingly, the previously reported elevations in QTVI in individuals with SCI compared to the able-bodied[11,15] are likely due to autonomic imbalances rather than end organ pathology[11]. In comparison to these earlier studies, QTVI in the current study was not different between AB individuals and those with autonomically incomplete SCI during supine rest. The fact that there were no differences in responses between the groups may very well be confounded by the inability to detect a difference between two groups with such a low n and low statistical power. However, another postulate is that although autonomic physiology is altered following incomplete SCI, there may not be a difference in QTVI responses between AB individuals and those with autonomically incomplete SCI. This postulate is supported by results from Ravensbergen et al. (2012) who demonstrated that individuals with autonomically incomplete SCI and AB controls have similar QTVI responses[15]. The preservation of cardiac autonomic function in the current SCI cohort was evidenced by the SCI participants showing a relative maintenance of blood pressure during the CV stress condition, and the HR responses to the different conditions were similar in both groups (Table 3). This suggests intact central command of cardiovascular functions in the SCI, which points at maintenance of autonomic integrity.

However, larger scale invasive studies are warranted to confirm that QTVI responses are similar between AB and autonomically incomplete SCI because the only available evidence for this speculation is from studies during resting conditions only (Ravensbergen et al., 2012)[15] and low sample size (current investigation).

Currently, HRV plays an important role in evaluating cardiac autonomic activity, and can identify autonomic responsiveness in AB and SCI individuals under different autonomic states[19]. The current study is a further analysis of previously collected ECGs, by our group, that evaluated HRV as an index of cardiac autonomic function in individuals with incomplete SCI[19]. Specifically, we showed from frequency domain analysis of HRV that HF power was almost completely abolished after atropine administration, suggesting its role as a surrogate for cardiac parasympathetic outflow. Further, metoprolol administration during CVS resulted in LF:HF ratio reduction, suggesting that the ratio may be representative of cardiac sympathovagal balance in individuals with incomplete SCI. It is clear from the current investigation that the QTVI is also influenced by similar alterations in autonomic activity, however, it is still uncertain which method better reflects cardiac autonomic activity in individuals with incomplete SCI, and warrants further investigation. Results from this study are in agreement with previous work suggesting that QTVI is an index of sympathetic activity at times of elevated CV stress. This was shown by the increase in QTVI during CV stress and its subsequent reduction after metoprolol administration. It is possible that the increase in QTVI that was seen when moving from the supine position to CV stress was due to vagal withdrawal, rather than sympathetic activation. However, this is unlikely, as subsequent β-blockade reduced the QTVI back to values that were similar to baseline.

Thus, during times of autonomic arousal, increases in the QTVI are more reflective of sympathetic activity per se, rather than vagal withdrawal. In agreement with these findings, a recent report by Baumert et al. (2011) showed elevated QT variability only in hypertensive patients, who have increased sympathetic tone, but not in healthy resting subjects[11]. Similarly, Piccirillo et al. (2009) found no correlation between QTVI and integrated stellate ganglionic nerve activity in dogs at baseline, but after inducing congestive heart failure, QTVI and stellate ganglionic nerve activity were significantly correlated. These reports, in addition to the current study, agree that QTVI seems to reflect cardiac sympathetic outflow during times of elevated sympathetic activity[10].

The current investigation also suggests that QTVI may be inversely related to cardiac vagal outflow, but only at times of rest. This was evidenced by an increase in QTVI during supine rest after administration of atropine. These results are in agreement with previous findings from Piccirillo et al. (2009), who demonstrated a negative correlation between QTVI and integrated vagal nerve activity in dogs at rest[10]. However, after inducing congestive heart failure, there was no correlation between the two variables. Further, in a recent study on individuals with SCI, La Fountaine et al. (2011) reported elevated QTVI values in individuals with tetraplegia compared to able-bodied controls during supine resting condition, which was attributed to an attenuated cardiac autonomic outflow in the SCI group[11]. Furthermore, the authors found a negative correlation between HF power and QTVI, and there is strong evidence that HF power is a valid index of parasympathetic cardiac control after SCI[19,25,26]. Therefore, it appears that QTVI is influenced by both sympathetic and parasympathetic outflow to the heart, depending on the dominating system. As stated by Berger (2009) with regards to

autonomic influence on QTVI, when multiple factors influence a physiological variable, the correlation between any one input and the output variable is highest when the strength of that input rises above all others, and fades when that input becomes overwhelmed by the others[27]. Accordingly, when the parasympathetic nervous system is dominant (at rest), it is inversely reflected by the QTVI, but when the sympathetic nervous system is dominant at times of CV stress, it is directly reflected by the QTVI. It is also important to note that although the QTVI is undoubtedly influenced by cardiac autonomic function in individuals with incomplete SCI, it is not necessarily a clean measure of cardiac autonomics, as it is still unknown if the QTVI is influenced equally by cardiac sympathetic and parasympathetic activity. Therefore, further studies are warranted in order to elucidate the ability of the QTVI to quantify cardiac sympathetic and parasympathetic activity in individuals with SCI.

Because the QT interval is influenced by HR, the RR-interval is also a major determinant in the QTVI calculation. Accordingly, the QTVI can increase from either increases in QT variability, or decreases in RR variability. Results from the current study show that during the sympathetically induced increase in QTVI, there was a concomitant increase in QTv but no change in RRv. In contrast, during the resting supine conditions, RRv significantly decreased in response to atropine administration while no change occurred in QTv. This suggests that sympathetic influence on QTVI may occur through increasing QT variability, whereas vagal influence on QTVI may occur through decreasing HR variability. This is in agreement with findings from Piccirillo et al. (2009) who showed that during resting conditions, there was a negative correlation between RRv and cardiac vagal outflow. After inducing congestive heart failure however, there was a

strong relationship between QTv and integrated stellate ganglionic nerve activity[10]. Similarly, Mine et al. (2008) showed a decrease in QT variability after propranolol administration with no change in RR interval[21]. The authors reported that sympathetic influence on QT variability does not require changes in the RR interval, and may occur by an alternate, more direct mechanism, such as QT variability. Taken together, these reports suggest that at rest, vagal modulation of QTVI may occur through changes in HR, while sympathetic activity influences QTVI through changes in QT variability.

## Limitations:

The main limitation to the current study is the small sample size and high variability within participants, however, all participants responded similarly to the autonomic blockade in terms of its influence on QTVI. In addition, the fact that there were no significant differences in QTVI between groups may be due to the low sample size. Therefore, larger scale studies are warranted to confirm these results. The second limitation to the study was that the order of drug administration was not randomized and only one order of drug administration was used (metoprolol followed by atropine). Therefore, we cannot comment on the effects of atropine alone on QTVI. Due to the preliminary nature of this study, and the invasiveness of the testing, the authors felt that a second day of drug administration was not justified. However, in the supine position, the administration of metoprolol has no significant effect on the QTVI, and thus, it did not likely confound the effects observed with subsequent atropine administration. Still, further research is required to shed light on this issue. The third limitation to the study was the high range of years post injury, however, there is no current evidence suggesting a degenerative/progressive change in cardiac autonomic regulation during the chronic

stages of incomplete SCI, and therefore the wide range of years post injury was likely not a confounder. Another limitation to the study was that only individuals with autonomically incomplete SCI were included, and therefore, these results cannot be generalized to all SCI cases, as those with complete loss of autonomic control are likely to show an attenuated response to CVS and thus, would provide great insight on the value of QTVI in the SCI population. However, a large portion of the SCI population includes those with incomplete injuries, therefore, the results from the current are clinically relevant for many individuals with SCI.

## Conclusions:

The QTVI appears to be influenced by both sympathetic and parasympathetic cardiac regulation depending on the state. At times of elevated CV stress, the QTVI seems to reflect the increase in sympathetic outflow, whereas during resting conditions, the QTVI seems to be inversely reflective of vagal outflow. Further, the relationship between QTVI and autonomic cardiac control appears to be maintained after autonomically incomplete SCI.

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## References:


1) Berger RD, Kasper EK, Baughman KL, Marban, R, Calkins H, Tomaselli GF: Beat-to-beat QT interval variability. Novel evidence for repolarization lability in ischemic and nonischemic dilated cardiomyopathy. Circulation 1997, 96:1557-65.

2) Atiga WL, Calkins H, Lawrence J, Tomaselli GF, Smith JS, Berger RD: Beat-to-beat repolarization lability identifies patients at risk for sudden cardiac death. J. Cardiovasc Electrophysiol 1998, 9:899-908.

3) Piccirillo G, Germano G, Quaglione R, Nocco M, Lintas F, Lionetti M, Moise A, Ragazzo M, Marigliano V, Cacciafesta M: QT-interval variability and autonomic control in hypertensive subjects with left ventricular hypertrophy. Clin.Sci 2002; 102:363-371.

4) Baumert M, Schlaich PM, Nalivaiko E, Lambert E, Sari CI, Kaye DM, Elser MD, Sanders P, Lambert G: Relation between QT interval variability and cardiac sympathetic activity in hypertension. Am J Physiol Heart Circ Physiol 2011; 300:H1412–H1417.

5) Raughunandan DS, Desai N, Mallavarapu M, Berger RD, Yeragani VK: Increased beat-to-beat QT variability in patients with congestive cardiac failure. Indian Heart J 2005; 57:138-42.

6) Piccirillo G, Magnanti M, Matera S, Carlo SD, Laurentis TD, Torrini A, Marchitto N, Ricci R, Margi D: Age and QT variability index during free breathing, controlled breathing and tilt in patients with chronic heart failure and healthy control subjects. Transl Res 2006; 148:72-8.

7) Baumert M, Smith J, Catcheside P, McEvoy RD, Abbott D, Sanders P, Nalivaiko E: Variability of QT interval duration in obstructive sleep apnea: An indicator of disease severity. Sleep 2008; 31:959-966.

8) Yeragani VK, Pohl R, Jampala VC, Balon R, Ramesh CM, Srinivasan K: Increased QT variability in patients with panic disorder and depression. Psychiatry Res 2000a; 93:225-35.

9) Yeragani VK, Pohl R, Jampala VC, Balon R, Kay J, Igel G: Effect of posture and isoproterenol on beat-to-beat heart rate and qt variability. Neuropsychology 2000b; 41:113-23.

10) Piccirillo G, Magri D, Ogawa M, Song J, Chong VJ, Han S, Joung B, Choi EK, Hwang S, Chen LS, Lin SF, Chen PS: Autonomic nervous system activity measured directly and qt interval variability in normal and pacing-induced tachycardia heart failure dogs. J Am Coll Cardiol 2009; 54:40-850.

11) La Fountaine MF, Wecht JM, Rosado-Rivera D, Cirnigliaro CM, Spungen AM, Bauman WA: The QT variability index and cardiac autonomic modulation: perspectives from apparently healthy men with spinal cord injury. Cardiology 2011; 117:253-9.

12) Grimm DR, De Meersman RE, Almenoff PL, Spungen AM, Bauman WA: Sympathovagal balance of the heart in subjects with spinal cord injury Am J Physiol 1997; 272:H835–H842.

13) Teasell RW, Arnold JM, Krassioukov A, Delaney GA: Cardiovascular consequences of loss of supraspinal sympathetic nervous system after spinal cord injury. Arch Phys Med Rehabil 2000; 81:506-16.

14) Valles M, Benito J, Portell E, Vidal J: Cerebral hemorrhage due to autonomic dysreflexia in a spinal cord injury patient. Spinal Cord 2005; 43:738-40.

15) Ravensbergen HJ, Walsh ML, Krassioukov AV, Claydeon VE: Electrocardiogram-based predictors for arrhythmia after spinal cord injury. Clin Auton Res 2012; 22:265-73.

16) Grimm DR, DeMeersman RE, Garofano RP, Spungen AM, Bauman WA: Effect of provocative manoeuvres on heart rate variability in subjects with quadriplegia. Am J Physiol 1995; 268:2239–45.

17) Houtman S, Oeseburg B, Hughson RL, Hopman MT: Sympathetic nervous system activity and cardiovascular homeostasis during head-up tilt in patients with spinal cord injuries. Clin Auton Res 1998; 10:207–12.

18) Ditor DS, Kamath MV, Macdonald MJ, Bugaresti J, McCartney N, Hicks AL: Reproducibility of heart rate variability and blood pressure variability in individuals with spinal cord injury. Clin Auton Res 2005; 15:387-93.

19) Cotie LM, Amand T, McMillan R, Picton P, Ditor DS: Effects of autonomic blockade on heart rate variability after spinal cord injury: A case series. Crit Rev Phys Rehabil Med 2010; 22:1-11.

20) Malik M: Problems of heart rate correction in assessment of drug-induced QT interval prolongation. J Cardiovasc Electrophysiol 2001; 12:411-20.

21) Mine T, Shimizu H, Hiromoto K, Furukawa Y, Kanemori T, Nakamura K, Masuyama T, Ohyanagi M: Beat-to-beat QT interval variability is primarily affected by the autonomic nervous system. Ann Noninvasive Electrocardiol 2000; 13:228-33.

22) Haigney MC, Wei S, Kaab S, Griffiths E, Berger R, Tunin R, Kass D, Fisher WG, Silver B, Silverman H: Loss of cardiac magnesium in experimental heart failure

prolongs and destabilizes repolarization in dogs. J Am Coll Cardiol 1998; 31:701–706.

23) Akar FG, Rosenbaum DS: Transmural electrophysiological heterogeneities underlying arrhythmogenesis in heart failure. Circ. Res 2003; 93:638-45.

24) Dobson GP, Kim A, Haigney M: QT Variability Index. Progres Cardiovasc Dis 2013; 56:186-94.

25) Koh, J., Brown, T.E., Beightol, L.A., Ha, C.Y., Eckberg, D.L., 1994. Human autonomic rhythms: vagal cardiac mechanisms in tetraplegic subjects. J. Physiol. 474, 483-495.

26) Millar PJ, Cotie LM, Amand T, McCartney N, Ditor DS: Effects of autonomic blockade on non-linear heart rate dynamics. Clin Auton Res 2010; 20:241-247.

27) Berger RD: QT interval variability. Is it a measure of autonomic activity? J Am Coll Cardiol 2009; 54:851-852.

**Chapter 5**
**Manuscript 2**

# Reproducibility of the QT-Variability Index in Individuals with Spinal Cord Injury

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**Abstract:**

**Purpose:** To examine the day-to-day reproducibility of the QT-variability index (QTVI) and the QT-apex variability index (QTaVI) in individuals with spinal cord injury (SCI).

**Methods:** Ten individuals with SCI participated in the current study (C2-T10; AIS A-D; 8.6 ± 7.8 years post injury). On two occasions, with a 10-day interval, a 10-minute resting electrocardiogram was obtained from each participant. The QTVI and QTaVI were analyzed from 256 electrocardiographic beats from all participants, and a separate analysis was performed on those with injuries above the 4$^{th}$ thoracic level. An intraclass correlation coefficient (ICC) test was performed to measure day-to-day reproducibility of these measures and a Bland-Altman test was performed on all participants in order to examine the skewness of the measures.

**Results:** The reproducibility values were found to be high for both the QTVI (all participants: R=0.892; above T4: R=0.893) and the QTaVI (all participants: R=0.908; above T4: R=0.915). In addition, the reproducibility of QTVI and QTaVI did not appear to be skewed as indicated by Bland-Altman plots.

**Conclusion:** Both the QTVI and the QTaVI may be used as reproducible means of assessing cardiac autonomic function in individuals with SCI. Further, a reduction in cardiac sympathetic regulation after high thoracic and cervical level SCI does not appear to influence the day-to-day reproducibility of these measures.

**Keywords:** Spinal cord injury, electrocardiogram, autonomic function, QT-interval.

## Introduction:

It is well documented that spinal cord injury (SCI) results in complete or partial loss of supraspinal control of the cardiovascular system, which therefore increases the risk for cardiovascular dysfunctions and mortality[1]. Specifically, injuries above the 5$^{th}$ thoracic level (T5) may lead to compromised cardiac sympathetic innervation, which increases the susceptibility to cardiac arrhythmias[2-4] and reduces exercise tolerance[5]. In addition, studies have reported a reduction in cardiac parasympathetic outflow following SCI, believed to be partly attributed to a physiological adaptation in an attempt to maintain sympathovagal balance[6,7]. However, such a reduction in cardiac vagal outflow may be problematic, as it has been demonstrated through a wide range of indicators that reduced vagal activity is associated with increased risk of mortality and morbidity, independent of traditional risk factors[8]. Furthermore, the loss of supraspinal control over sympathetic preganglionic neurons that innervate the vascular bed is associated with both autonomic dysreflexia (characterized by episodic fluctuations in blood pressure)[9] and orthostatic hypotension, which may reduce functional capacity and the ability to engage in activities of daily living. Thus, it is imperative to develop reliable, non-invasive measures of cardiac autonomic activity in individuals with SCI in order to assess the integrity of cardiac autonomic regulation and potential changes over time.

Several electrocardiogram (ECG)-based measures have been employed for non-invasive assessment of cardiac autonomic regulation after SCI, including spectral analysis of heart rate variability (HRV) and non-linear HRV. Although both measures have been shown to be reproducible in individuals with SCI[10,11], not all parameters achieve that status, as high frequency power was shown to have poor reproducibility in this

population[10]. In addition, the efficacy of some of these parameters in gauging cardiac sympathetic activity in the SCI population remains questionable[12,13]. More recently however, the QT-variability index (QTVI) has been developed as a non-invasive measure of beat-by-beat repolarization variability[14]. This measure takes simultaneous QT and RR intervals into consideration, thus providing insight on both myocardial (QT-interval) and autonomic (RR-interval) activity. An elevation in QTVI suggests more repolarization variability, while a reduction in QTVI suggests more stable and less variable repolarization time. An increase in QTVI has been strongly associated with future cardiac events[15,16], hypertension[17], cardiac arrhythmias[18] and heart failure[19]. In addition, it is also augmented in non-pathological settings that are associated with changes in cardiac autonomic activity, such as aging[20] and exercise[21]. In addition, the QTVI has been shown to be moderately reproducible in healthy humans[22] and patients with kidney failure[23].

The QTVI has been shown to be elevated in individuals with SCI[24], especially those with injuries above T5 and/autonomically complete injuries[4]. A recent study from our laboratory has demonstrated the QTVI's ability to reflect both cardiac sympathetic and parasympathetic activity in individuals with tetraplegia[25]. Although the QTVI is a relatively novel method, it may be a promising method for gauging the amount of preserved cardiac autonomic function in individuals with SCI. However, autonomic decentralization following SCI may cause the cardiovascular system to operate on reflexes alone, rather than well regulated supraspinal signals. Hence, because the QTVI is highly influenced by cardiac autonomic activity[20,26], autonomic decentralization may render the QTVI unstable. Therefore, before the QTVI can be effectively employed as a measure of cardiac autonomic activity in individuals with SCI, its reproducibility must be

examined in this population. Accordingly, the purpose of this study was to examine the day-to-day reproducibility of the QTVI in individuals with SCI.

**Methods:**

*Participants:*

Electrocardiographic data from 10 individuals (6 male, 4 female; age 44.8 ± 15.0 years) with SCI (C2-T10; AIS A-D; 8.6 ± 7.8 years post-injury) were collected and analyzed for this study. Participant characteristics can be found in Table 1. All participants were at least 1 year post injury and were recruited from a community based rehabilitation program for individuals with SCI (Power Cord, St. Catharines, Ontario). All participants were medically examined and had no existing cardiovascular disease or cardiac pacemakers, and other than the SCI, they were healthy and free from secondary health compilations.

**Table 1: Participant Characteristics**

| Participant | Gender | Age | Injury Level | AIS Classification | Years Post Injury |
|---|---|---|---|---|---|
| 1 | M | 21 | C7 | B | 4 |
| 2 | F | 37 | T3 | A | 18 |
| 3 | M | 41 | C6 | B | 5 |
| 4 | M | 65 | C5 | D | 5 |
| 5 | M | 67 | C2 | B | 3 |
| 6 | F | 43 | C7 | C | 9 |
| 7 | F | 45 | C7 | A | 8 |
| 8 | F | 27 | T6 | A | 6 |
| 9 | M | 44 | T6 | A | 27 |
| 10 | M | 58 | T10 | A | 5 |

AIS: ASIA Impairment Scale, where ASIA stands for the American Spinal Injury Association.

*Study Protocol:*

Each participant visited the laboratory on two occasions (Day 1 and Day 2) within a 10-day period, and no two testing sessions were less than seven days apart. All testing sessions took place between the hours of 12pm and 4pm, and each participant was tested at the same time of day during both visits. Participants were asked to refrain from any alcohol consumption or smoking 24 hours prior to testing and to avoid caffeine intake the morning of testing. In addition, any types of exercise, aside from morning stretches, were to be avoided 24 hours prior to testing and each participant was at least 1 hour post-prandial during data collection. Medications were not discontinued during data collection, however, participants were instructed to maintain the same dosage during both days of testing. Medications that participants were on during data collection included baclofen, senokot, midodrine and fludrocortisone. All participants were asked to empty their urine bags, bladders or bowels before arriving to the laboratory.

Upon arriving to the laboratory, participants were transferred onto a plinth and a single lead ECG (lead 2) was connected. Before data collection took place, the lights in the laboratory were dimmed and each participant was asked to lie down quietly in the supine position for 10 minutes in order for the participants to be in an autonomically relaxed state. Following 10 minutes of rest, 10 minutes of continuous ECG recordings were collected for later analysis of the QTVI (Power Lab, Lab Chart 7, ADInstruments). All ECG data were collected at a sampling frequency of 1000Hz and a band-pass filter of 0.5-50Hz was used in order to eliminate both high frequency noise and baseline wander, which can influence QT interval analysis.

*QT Interval Analysis:*

Two hundred and fifty six stable artifact free beats were used for QTVI analysis for each participant. The QT interval was analyzed in 2 ways: i) from the onset of the Q wave to the intersection point of the isoelectric line and the tangent line down the descending limb of the T-wave, and ii) from the onset of the Q wave to the apex of the T-wave. The latter analysis, known as QTaVI[17] is easier to detect and is less corrupted by noise compared to the end of the T-wave. The QTaVI method has been employed in assessing beat-to-beat repolarization variability in able-bodied[27] and SCI individuals[28].

Prior to further analysis, each beat was visually examined in order to ensure that the software marked the QT and QT-apex intervals appropriately. The QTVI was calculated using the following formula developed by Berger et al[14]:

$$QTVI = Log_{10} [(QTv/QTm^2) / (RRv/RRm^2)]$$

where, QTv is the QT interval variance, $QTm^2$ is the mean QT interval squared, RRv is the RR interval variability and $RRm^2$ is the mean RR interval squared.

The QTaVI was calculated using the formula:

$$Log_{10} [(QTav/QTam^2) / (RRv/RRm^2)]$$

where, QTav is the QT-apex interval variance and $QTam^2$ is the QT-apex interval mean squared, RRv is the RR interval variability and $RRm^2$ is the mean RR interval squared.

*Statistical Analysis:*

A dependent sample *t* test was performed on all participants (n=10) in order to compare Day1 and Day2 values for QTVI, QTaVI, RR interval and variability, QT interval and variability, as well as QT-apex interval and variability. Statistical significance was set at $p < 0.05$ and data are reported as means ± standard deviations. To

calculate the test:retest reproducibility of QTVI and QTaVI, 2-way mixed model intra-class correlations (ICC) were performed for all participants (n=10). Separate ICC's were also calculated for participants with injury levels above T4 (n=7), as such injuries are associated with a greater loss of control over cardiac function due to compromised cardiac sympathetic innervation. In addition, Bland-Altman plots were performed for QTVI and QTaVI in order to assess possible skewness of the data, ie, to determine if day to day differences depend on the magnitude of the QTVI or QTaVI values. All statistical analysis was performed using SPSS version 20 (SPSS Inc, Chicago).

## Results:

*QTVI and QTaVI Measures:*

The *t* test revealed no significant differences between Day 1 and 2 values for QTVI (-1.38±0.62 vs. -1.37±0.57; p=0.93) and QTaVI (-1.33±0.48 vs. -1.24±0.38; p=0.33). Intraclass correlation coefficients showed high reproducibility across all subjects for both QTVI and QTaVI (Table 2). When assessing only participants with injury level above T4, ICC's were also very high for QTVI and QTaVI (Table 2).

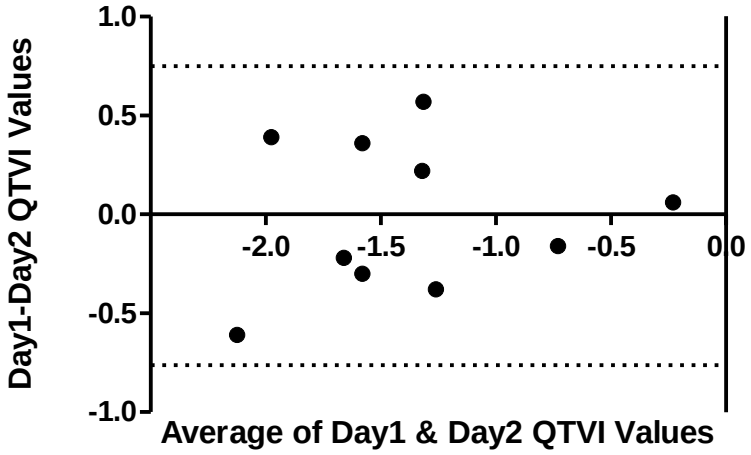
**Table 2: Reproducibility of QTVI and QTaVI**

| | **Day 1** | **Day 2** | **R** |
|---|---|---|---|
| All participants (n=10) | | | |
| QTVI | -1.38±0.62 | -1.37±0.57 | 0.892* |
| QTaVI | -1.33±0.48 | -1.24±0.38 | 0.893* |
| Injury Above T4 (n=7) | | | |
| QTVI | -1.35±0.73 | -1.19±0.57 | 0.908* |
| QTaVI | -1.31±0.58 | -1.18±0.43 | 0.915* |

QTVI: QT variability index; QTaVI: QT- apex variability index; R Intraclass correlation coefficient. Day 1 and Day 2 are expressed as means ± S.D. *denotes significant intraclass correlation ($p < 0.05$).

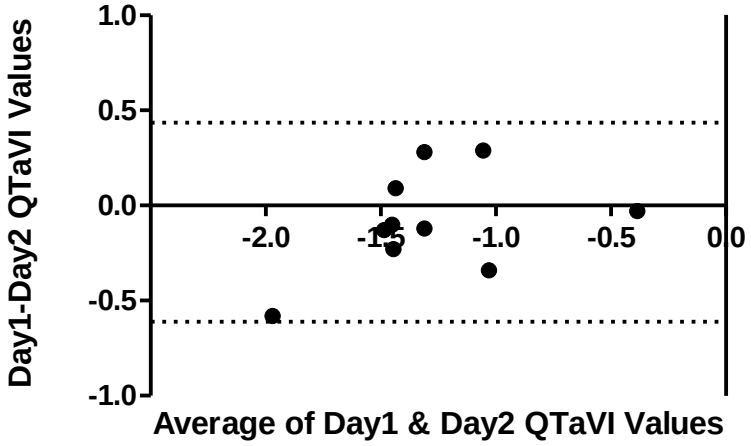
The reproducibility of QTVI and QTaVI did not appear to be skewed as indicated by Bland-Altman plots (Figures 1 & 2). Thus, the reproducibility of these measures do not appear to depend on the actual value of the measure.

**Figure 1: Bland-Altman Plot for QTVI**




QTVI: QT-variability index

**Figure 2: Bland-Altman Plot for QTaVI**




QTaVI: QT-apex variability index

T-tests were also performed to compare Day 1 and Day 2 values for RR interval, RR variability, QT interval, QT variability, QTa interval and QTa variability and the results showed no significant differences between the two days for any of these variables (Table 3).

**Table 3. Day to day values for electrocardiographic parameters**

| | Day 1 | Day 2 | p-Value |
|---|---|---|---|
| RR interval (ms) | 1005.88±144.8 | 1002±115.08 | 0.93 |
| RR Variance | 2362.54±1662.73 | 2327.24±2073.86 | 0.95 |
| QT Interval (ms) | 372.31±25.43 | 373.81±23.74 | 0.80 |
| QT Variance | 13.77±14.33 | 9.90±5.65 | 0.41 |
| QTa Interval(ms) | 309.38±25.07 | 310.63±21.95 | 0.81 |
| QTa Variance | 10.72±13.17 | 8.87±3.74 | 0.65 |

QTa: QT-apex interval.

**Discussion:**

The main finding of the current study is that both QTVI and QTaVI were found to be highly reproducible in individuals with SCI. The reproducibility was evident when all participants were considered as a whole, as well as when individuals with injury levels above T4 were analyzed separately. Therefore, it appears that spinal lesions that may compromise cardiac sympathetic outflow do not adversely influence the reproducibility of the QTVI and QTaVI. Previous investigators have suggested ICC to be considered "good" if it ranged between 0.60 and 0.81 and almost perfect if exceed 0.81[29], while other investigators have suggested values need be a minimum of 0.81 to be considered

trustworthy[30]. The ICC values of the present study exceeded these suggested values with correlation coefficients ranging from 0.89 to 0.91.

Cardiovascular disease is a growing concern in the SCI population as it is the leading cause of mortality in this population[1]. The onset of cardiovascular dysfunctions manifests much earlier in the SCI population compared to able-bodied individuals and progresses at a more rapid rate. Loss of or blunted cardiovascular autonomic control following SCI is a possible explanation for the elevated risk for cardiovascular dysfunctions in this population. For example, injuries above the mid thoracic region have been associated with a reduction in both cardiac sympathetic and parasympathetic outflow[6]. The former is a result of disruption in the supraspinal cardiac sympathetic modulation, while the latter is likely a result of the profound physical inactivity associated with SCI. Furthermore, the reduced supraspinal control of sympathetic activity may result in potentially dangerous autonomic conditions such as autonomic dysreflexia, orthostatic hypotension and cardiac arrhythmias. Accordingly, there is a need for reliable methods to gauge cardiac sympathetic and parasympathetic integrity as a way to determine how much damage has been sustained to the autonomic nervous system and how much it improves or changes over time.

One such measurement tool, the QTVI, has been shown to reflect cardiac autonomic activity in several clinical populations[14,17], and healthy individuals[26]. In addition, individuals with SCI have been reported to exhibit higher QTVI values compared to healthy able bodied individuals[4,26]. Recently, our group demonstrated that QTVI may reflect both cardiac sympathetic and parasympathetic activity in individuals with autonomically incomplete injuries[25]. The ICC values for both the QTVI and QTaVI

obtained from the current study were shown to be higher than previously reported values for HRV in individuals with SCI. Ditor et al. reported ICC scores of 0.84 and 0.82 for low frequency and low to high frequency ratio, respectively[10]. Although it is not clear as to why QTVI seems to be more reproducible than HRV, it could be because the QTVI is influenced by 2 factors: QT variance (numerator) and RR variance (denominator) (see formula). As such, if QTVI were to increase, this could be due to either an elevation in QT variance or a reduction in RR variance. Thus, if RR variability is subjected to day-to-day changes[10], this could be countered by the stability of QT variance. It is worth noting that non-linear measures of HRV, which are only derived from the RR interval, have produced higher ICC values compared to the QTVI from the current investigation[11]. However, the efficacy for non-linear HRV to reflect cardiac autonomic function in individuals with SCI is unclear[13], whereas the QTVI may hold more promise in that aspect[25].

Previous investigations reported moderate reproducibility for QTVI in healthy individuals[22] and patients with kidney failure[23]. It is interesting to note that although renal failure patients had significantly higher QTVI values compared to their healthy counterparts, both groups showed comparable reproducibility[23]. This suggests that the amount of ventricular repolarization variability per se may not influence QTVI reproducibility. Reproducibility of the QTVI may be more influenced by methodological techniques of measuring the QT-interval rather than physiological factors. Unpublished data from our laboratory suggest that the method of analyzing the QT-interval has a significant effect on the QTVI. In addition, Gao et al. (2005) reported that their results may be influenced by the algorithm used, as it was difficult to properly define QT-

intervals on morphologically abnormal ECGs[23]. However, further research with a bigger sample size is warranted to confirm our results of highly reproducible QTVI in the SCI population.

**Conclusion:**

This is the first study to demonstrate that QTVI and QTaVI are reproducible in individuals with SCI, and this high degree of reproducibility also persists in individuals with injury levels above T4. Therefore, measures of QTVI should be employed in future research as a means of assessing cardiac autonomic function in individuals with SCI.

**Ethical Standards:**

This study was approved by the Brock Biosciences Research Ethics Committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All individuals gave their informed consent prior to their inclusion in the study.

**Conflict of Interest:**

The authors have no conflict of interest.

**References:**


1. Myers J, Lee M: Cardiovascular Disease in Spinal Cord Injury. Am J Phys Med Rehabil 2007; 86:142–52.

2. Collins HL, Rodenbaugh DW, DiCarlo SE: Spinal cord injury alters cardiac electrophysiology and increases the susceptibility to ventricular arrhythmias. Prog Brain Res 2006;152: 275-88.

3. Lujan HL, Palani G, DiCarlo SE: Structural neuroplasticity following T5 spinal cord transection: increased cardiac sympathetic innervation density and SPN arborization. Am J Physiol Regul Integr Comp Physiol 2010; 299:R985-R995.

4. Ravensbergen HJ, Walsh ML, Krassioukov AV, Claydon VE: Electrocardiogram-based predictors for arrhythmia after spinal cord injury. Clin Auton Res 2012; 22:265–73.

5. Eriksson P, Löfström L, Ekblom B: Aerobic power during maximal exercise in untrained and well-trained persons with quadriplegia and paraplegia. Scand J Rehabil Med 1998; 20:141–7.

6. Grimm DR, De Meersman RE, Almenoff PL, Spungen AM, Bauman WA: Sympathovagal balance of the heart in subjects with spinal cord injury Sympathovagal balance with spinal cord injury of the heart in subjects. Am J Physiol 1997; 272:H835–H842.

7. Wang YH, Huang TS, Lin JL, Hwang JJ, Chan HL, Lai JS, et al: Decreased autonomic nervous system activity as assessed by heart rate variability in patients with chronic tetraplegia. Arch Phys Med Rehabil 2000; 81:1181–4.

8. Thayer JF, Lane RD: The role of vagal function in the risk of cardiovascular disease and mortality. Biol Psych 2007; 74:224-42.

9. Karlsson AK: Autonomic Dysreflexia. Spinal Cord 1999; 37:383-91.

10. Ditor DS, Kamath MV, MacDonald MJ, Bugaresti J, McCartney N, Hicks AL: Reproducibility of heart rate variability and blood pressure variability in individuals with spinal cord injury. Clin Auton Res 2005; 15:387-93.

11. La Fountaine MF, Wecht JM., Spungen AM, Bauman WA: Intra-inter visit reproducibility of short-term linear and nonlinear measurement of heart rate variability in tetraplegia and neurologically intact controls. Physiol Meas 2010; 31:363–74.

12. Cotie LM, St. Amand T, McMilan R, Picton P, Ditor DS: Effects of autonomic blockade on heart rate variability after spinal cord injury: A case series. Crit Rev Phys Rehabil Med 2010; 22:1-12.

13. Millar PJ, Cotie LM, St Amand T, McCartney N, Ditor DS: Effects of autonomic blockade on nonlinear heart rate dynamics. Clin Auton Res 2010; 20:241–7.

14. Berger RD, Kasper EK, Baughman KL, Marban R, Calkins H, Tomaselli GF: Beat-to-beat QT interval variability. Novel evidence for repolarization lability in ischemic and nonischemic dilated cardiomyopathy. Circulation 1997; 96:1557-65.

15. Atiga WL, Calkins H, Lawrence J, Tomaselli GF, Smith JS, Berger RD: Beat-to-beat repolarization lability identifies patients at risk for sudden cardiac death. J Cardiovasc Electrophysiol 1998; 9:899-908.

16. Tereshchenko LG, Cygankiewicz I, McNitt S, Vasquez R, Bayes Genis A, et al: Predictive value of beat-to-beat qt variability index across the continuum of left ventricular dysfunction: competing risks of noncardiac or cardiovascular death and sudden or non-sudden cardiac death. Circ Arrhythm Electrophysiol 2012; 5:719-27.

17. Baumert M, Schlaich PM, Nalivaiko E, Lambert E, Sari CI, Kaye DM: Relation between QT interval variability and cardiac sympathetic activity in hypertension. Am J Physiol Heart Circ Physiol 2011; 300:H1412–H1417.

18. Oosterhoff P, Tereshchenko LG, van der Heyden MA, Ghanem RN, Fetics BJ, Berger RD, et al: Short-term variability of repolarization predicts ventricular tachycardia and sudden cardiac death in patients with structural heart disease: a comparison with QT variability index. Heart Rhythm 2011; 10:84-1590.

19. Piccirillo G, Magnanti M, Matera S, Carlo SD, Laurentis TD, Torrini A, et al: Age and QT variability index during free breathing, controlled breathing and tilt in patients with chronic heart failure and healthy control subjects. Transl Res 2006; 148:72-80.

20. Piccirillo G, Magrì D, Ogawa M, Song J, Chong VJ, Han S, et al: Autonomic nervous system activity measured directly and QT interval variability in normal and pacing-induced tachycardia heart failure dogs. J Am Coll Cardiol 2009; 54:840–50.

21. Boettger S, Puta C, Yeragani VK, Donath L, Müller HJ, Gabriel HH, et al: Heart rate variability, QT variability, and electrodermal activity during exercise. Med Sci Sports Exerc 2010; 42:443-8.

22. Starc, V, Abughazaleh AS, Schlegel TT: Reliability and reproducibility of advanced ECG parameters in month-to-month and year-to-year recordings in

healthy subjects. In Cardiovascular Oscillations (ESGCO), 2014 8th Conference of the European Study Group on (pp. 55-56). IEEE.

23. Gao SA, Johansson M, Hammarén A, Nordberg M, Friberg P: Reproducibility of methods for assessing baroreflex sensitivity and temporal QT variability in end-stage renal disease and healthy subjects. Clinic Auton Res 2005; 15:21-8.

24. La Fountaine MF, Wecht JM, Rosado-Rivera D, Cirnigliaro CM, Spungen AM, Bauman WA: The QT variability index and cardiac autonomic modulation: perspectives from apparently healthy men with spinal cord injury. Cardiology 2010; 117:253-9.

25. Sharif H, Cotie LM, La Fountaine MF, Ditor DS. The influence of cardiac autonomic activity on the QT-variability index in able-bodied and incomplete spinal cord injured individuals. Auton Neurosci 2015; 190:45-52.

26. Yeragani VK, Pohl R, Jampala VC, et al: Effect of posture and isoproterenol on beat-to-beat heart rate and QT variability. Neuropsychobiology 1999; 41:113-23.

27. Piccirillo G, Cacciafesta M, Lionetti M, et al: Influence of age, the autonomic nervous system and anxiety on QT-interval variability. Clin Sci 2001; 101:429-38.

28. La Fountaine MF, Wecht JM, Cirnigliaro CM, et al: Testosterone replacement therapy improves QTaVI in hypogonadal men with spinal cord injury. Neuroendocrinology 2010; 97:341-6.

29. Landis JR, Koch GG: The measurement of observer agreement for categorical data. Biometrics 1977; 33:159–74.

30. Hopkins WG: Measures of reliability in sports medicine and science. Sports Med 2000; 30:1–15.

# Chapter 6

## Manuscript 4

## Cardiac Autonomic and Ventricular Diastolic Interactions in Individuals with Spinal Cord Injury

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**Abstract:**

**Objective:** To examine the relationship between resting cardiac autonomic function and left ventricular diastolic function in able-bodied and spinal cord injured (SCI) individuals. To determine the autonomic and diastolic responses to vagal stimulation via the cold face test (CFT) in both groups.

**Methods:** Thirteen SCI (Age: 41±8.5; 10 males, 3 females) and 12 able-boded (AB) individuals (Age: 40±8.5; 9 males, 3 females) without history of cardiovascular disease participated in the study. Baseline cardiac parasympathetic function was assessed from a 5-minute electrocardiographic (ECG) epoch via heart rate variability (HRV) and the QT-variability index (QTVI). This was followed by assessment of baseline LV ventricular function by conventional echocardiography. In order to assess autonomic responses to vagal stimulation, 1-minute ECG data was collected before and during the CFT, and in order to assess diastolic response to vagal stimulation, LV diastolic function was assessed immediately at the end of the CFT.

**Results:** At baseline, the able-bodied group showed strong correlations between early diastolic filling and cardiac parasympathetic activity, as shown by SDNN ($r=0.61$; $p=0.03$), RMSSD ($r=0.75$; $p=0.003$), coefficient of variance ($r=0.61$; $p=0.03$) and the QTVI ($r=-0.60$; $p=0.03$). These relationships however were absent in the SCI group, as there were no significant correlations between any of the parameters. During the CFT, the able-bodied group experienced a significant reduction in heart rate ($p<0.05$) as well as a significant increase in HRV ($p<0.05$). In contrast, the SCI group exhibited strong trends for elevated HRV, but no change in heart rate during the CFT ($p>0.05$). Moreover, the able-bodied group showed positive correlations between an increase in HRV and diastolic filling during the CFT ($p<0.05$), while the SCI group showed a positive correlation between an increase in HRV and deceleration time ($p<0.05$).

**Conclusion:** Parasympathetic activity plays a role in modulating diastolic function at rest, however, this relationship seems to be lost following SCI. In addition, the increase in cardiac vagal activity during the CFT results in atypical heart rate and diastolic responses in those with SCI.

**Introduction:**

Left ventricular (LV) diastolic function is governed by the combined effects of factors such as loading conditions[1,2], LV elasticity[3,4], LV morphology and cardiac autonomic function[5]. When all of these factors operate concomitantly in a harmonious fashion, the LV is able to rapidly and passively fill with blood during early diastole, and continue to fill with blood via atrial contraction during late diastole. Alterations in any of the aforementioned factors can disturb diastolic filling and thus, may increase the risk for further cardiac complications[5]. The autonomic nervous system plays a role in maintaining normal cardiac function, as it is well established that the heart is constantly operating under the balance of sympathetic and parasympathetic activity[6]. Although the literature on autonomic-diastolic interactions is scarce, there is evidence suggesting that cardiac autonomic function may play a role in orchestrating LV diastolic function[7-10]. Previous studies have shown that a reduction in indices of cardiac parasympathetic activity are correlated with diastolic impairments in patients with diabetes[11,12] and heart disease[13]. In addition, experimental animal studies have shown that LV diastolic impairments manifest at approximately the same time as when cardiac parasympathetic regulation begins to deteriorate[14]. Furthermore, studies in healthy humans have shown that individuals with lower diastolic function demonstrate lower values of heart rate variability (HRV)[15]. Finally, direct vagal stimulation in animals has been shown to increase diastolic filling in animals[16], whereas parasympathetic withdrawal in humans has been shown to reduce diastolic filling[7,10].

Spinal cord injury (SCI) is a condition well known to induce impairments in the autonomic regulation of the cardiovascular system. This is mainly attributed to the

reduction, or loss, of supraspinal control of descending sympathetic signals that modulate cardiovascular activity[17]. Although the vagus nerve is not directly influenced by SCI, as it exits from the brainstem, individuals with SCI still demonstrate alterations in cardiac parasympathetic activity. Non-invasive measures have shown that individuals with SCI demonstrate altered chronotropic responses to cardiac vagal activity[18,19]. For example, vagal withdrawal resulted in a blunted HR response in those with SCI[18], whereas non-invasive cardiac vagal stimulation resulted in paradoxical tachycardia[19]. It is unknown if such chronotropic impairments in response to autonomic activity extend to impairments in mechanical relaxation of the heart as well. However, since diastolic function appears to be influenced by cardiac parasympathetic activity, one could hypothesize that alterations in cardiac parasympathetic activity would result in altered diastolic function as well.

The cold face test (CFT) is an assessment based on the diving reflex, and is a method of assessing the integrity of the trigeminal-brainstem-vagal pathway. It is non-invasive, requires minimal active participant cooperation and is time efficient, thus making it a favorable autonomic test. The typical cardiac response to the CFT is pronounced bradycardia due to enhanced cardiac parasympathetic outflow[20,21]. Therefore, the CFT may be employed to non-invasively examine the interaction between changes in cardiac vagal activity and LV diastolic function in able-bodied and SCI individuals. Accordingly, this is an exploratory study aiming to examine 1) the relationship between resting cardiac parasympathetic activity and LV diastolic function in able-bodied and SCI individuals at rest, 2) and LV diastolic changes in response to cardiac parasympathetic stimulation via the CFT in able-bodied and SCI individuals.

**Methods:**

*Participants:*

Twelve able-bodied individuals and 13 individuals with SCI (C4-T5; AIS A-D; 16.5±9.7 years post injury) participated in this study. Both groups were matched for age, sex and body mass. None of the participants had a history of cardiovascular disease, and all had normal resting and exercise electrocardiograms (ECG). Participants from both groups included individuals with habitual activity ranges from sedentary to physically active and none were highly trained or competitive athletes. Participants provided informed consent to participate in the study, which was approved by the Brock University Biosciences Research Ethics Board.

*Study Protocol:*

All participants were requested to void their bladder and bowel prior to testing in order to reduce the risk of any sympathetic influence on the experiments. Body mass (kg) and height were measured and from this body mass index (BMI: $kg/m^2$) was calculated. All medications for individuals with SCI were taken as prescribed during the day of testing. Medications that the participants were taking included baclophen for muscle spasms as well as anti-diuretics and alpha agonists for hypotension. Participants were in the left lateral decubitus position during the pre-test resting period as well as throughout the testing as changes in body position can alter cardiac autonomic activity[22]. Participants first rested in the left lateral decubitus position in a quiet, dim room for 10 minutes while breathing at 15 breaths/min as paced with a metronome. Following 10 minutes of rest, 5 minutes of baseline ECG data were collected for later analysis of baseline cardiac parasympathetic activity. Following baseline ECG collection, LV diastolic function was

assessed via echocardiography. Continuous ECGs were then recorded immediately before (Pre-CFT) and during the CFT in order to assess changes in cardiac parasympathetic activity. The cardiac probe was placed on the apical window throughout the CFT in order to keep the 4-chamber apical view in sight for immediate assessment of diastolic function at the termination point of the CFT.

*Cardiac Autonomic Activity:*

Baseline cardiac parasympathetic activity was measured from the 5 minutes of ECG data collected prior to the baseline cardiac imaging. A Single-lead (lead II) ECG was used for the collection of continuous HR for later analysis of cardiac parasympathetic activity (Power Lab, Lab Chart 7, AD Instruments). The ECG signal was sampled at a frequency of 1000 Hz, and a band-pass filter of 0.5-50 Hz was applied in order to eliminate signal corruption by high frequency noise and baseline wander[23]. None of the participants showed any arrhythmias before, during, or after, the CFT. The ECG signal was analyzed for both frequency and time domain HRV indices of parasympathetic regulation. Frequency domain measures included the natural log of the high frequency component of HRV (HFln), while time domain measures included the standard deviation of continuous RR intervals (SDNN), the root mean square of differences between RR intervals (RMSSD) and coefficient of variance (CV). In addition, cardiac parasympathetic activity was measured via the QT-variability index (QTVI)[24], which was calculated in accordance to the Berger formula[25] (Berger et al., 1997):

$$QTVI = Log_{10} [(QTv/QTm^2) / (RRv/RRm^2)]$$

where, QTv is the QT interval variance, $QTm^2$ is the mean QT interval squared, RRv is the RR interval variance and $RRm^2$ is the mean RR interval squared.

*Echocardiography:*

All echocardiographic images were obtained using a commercially available ultrasound system (Vivid Q; GE Vingmed Ultrasound AS, Horten, Norway) with a 1.5-MHz phased-array transducer. All images were acquired by a single experienced sonographer and were stored for later offline analysis using a commercially available software (EchoPac version 6.0; GE Vingmed Ultrasound AS). Imaging was performed while participants were in the left lateral decubitus position at end expiration and 3 consecutive cycles were stored for later analysis. Measures of diastolic function were calculated from the average of the 3 consecutive heart cycles.

Pulse-wave Doppler was used to assess diastolic function according to the recommendation of the American Society of Echocardiography[26]. For mitral inflow velocity, from an apical 4-chamber view, a 4 mm sample volume was placed between the mitral valve leaflet tips during diastole. The Doppler velocity curves were digitized to obtain peak early (E) and late (A) mitral flow velocities, and their ratio (E:A) was calculated, where a higher ratio illustrates greater reliance on early diastole, and a lower ratio depicts greater reliance on atrial contraction. The descending limb of the E wave was used to measure deceleration time (DT).

*Cold Face Test:*

Packs of ice-water slush (1.2 C˚) were placed on the forehead and bilateral maxillary areas for 1 minute. Care was taken to avoid the eyes in order to prevent stimulation of the oculo-cardiac vagal reflex[27]. Participants were instructed to avoid holding their breath and to breathe as normally as possible throughout the test. Because

the test is generally uncomfortable, the participants were informed every 10 seconds about how much time remained and were verbally encouraged to keep breathing. In order to avoid the potential influence of autonomic excitement before the onset of the CFT, the Pre-CFT autonomic data were obtained from a 1-minute ECG recording collected 1 minute prior to the start of the CFT, and the CFT recordings were collected during the minute while the cold packs were in contact with the participant's face. Time and frequency domain HRV indices were used to assess changes in cardiac parasympathetic assessment during the CFT. However, the QTVI was not used to assess changes in parasympathetic activity during the CFT, as it was difficult to obtain clean ECG signals for QT-interval assessments during the CFT.

*Statistical Analysis:*

Unpaired t-tests were used to measure between-group differences in participant characteristics, as well as baseline cardiac autonomic and ventricular parameters. Pearson's correlations were used to assess potential relationships between baseline cardiac parasympathetic activity and LV diastolic function. A 2-way repeated measure analysis of variance (ANOVA) was used to detect any group (controls vs SCI) by condition (CFT) interactions for autonomic and diastolic function and a t-test was used as a post-hoc when interactions were found. Cohen's D was used to estimate effect sizes[28]. Change variables were calculated for cardiac autonomic and LV diastolic function indices and unpaired t-test was used to determine between group differences. Pearson's correlations were used to determine relationships between the change variables in response to CFT. In addition, a partial Pearson's correlation was used to determine the relationship between the change variables while controlling for the change in HR, as HR

has been shown to correlate with cardiac parasympathetic indices[29]. Data is presented as means ± standard deviation, and all statistical analyses were performed using Statistical Package for Social Sciences (SPSS 22) software. Statistical significance was set at $p \leq 0.05$.

**Results:**

*Participants:*

All participant characteristics are provided in Table 1. There were no between-group differences in age, body mass or BMI. All individuals with SCI were at least 1 year post injury.

**Table 1. Participant Characteristics**

| Participant | Age (years) | Sex | Body Mass(kg) | BMI(kg/m) | HR(bpm) | Level | AIS | YPI |
|---|---|---|---|---|---|---|---|---|
| **SCI** | | | | | | | | |
| 1 | 55 | M | 80.7 | 26.8 | 73 | C4 | A | 8 |
| 2 | 42 | M | 68.2 | 20.8 | 53 | C4 | C | 18 |
| 3 | 31 | F | 64.0 | 22.7 | 61 | C5 | B | 6 |
| 4 | 46 | M | 83.0 | 28.7 | 65 | T6 | A | 19 |
| 5 | 23 | M | 83.2 | 27.8 | 57 | C8 | B | 5 |
| 6 | 43 | M | 81.0 | 27.1 | 43 | C4 | A | 6 |
| 7 | 46 | F | 61.0 | 22.7 | 73 | C7 | A | 9 |
| 8 | 37 | M | 100.0 | 31.6 | 70 | C5 | B | 18 |
| 9 | 38 | M | 96.4 | 25.9 | 54 | C5 | C | 38 |
| 10 | 54 | M | 95.0 | 29.3 | 55 | C567 | D | 27 |
| 11 | 38 | F | 56.0 | 21.1 | 64 | T3 | A | 20 |
| 12 | 41 | M | 66.0 | 24.2 | 77 | T5 | A | 16 |
| 13 | 44 | M | 91.0 | 27.2 | 67 | T4 | A | 24 |
| AVE | 41.38 | | 78.9 | 25.8 | 62 | --- | --- | 16.5 |
| SD | 8 | | 14.6 | 3.3 | 9 | --- | --- | 9.7 |
| **AB** | | | | | | | | |
| 1 | 44 | M | 94.3 | 26.3 | 50 | --- | --- | --- |
| 2 | 44 | M | 70.1 | 24.8 | 47 | --- | --- | --- |
| 3 | 35 | F | 57.2 | 21.2 | 75 | --- | --- | --- |
| 4 | 35 | M | 76.0 | 24.5 | 62 | --- | --- | --- |
| 5 | 41 | M | 102.0 | 30.5 | 51 | --- | --- | --- |
| 6 | 40 | M | 67.6 | 23.7 | 43 | --- | --- | --- |
| 7 | 30 | F | 57.4 | 20.9 | 63 | --- | --- | --- |
| 8 | 33 | M | 91.1 | 27.8 | 51 | --- | --- | --- |
| 9 | 54 | M | 102.1 | 27.4 | 62 | --- | --- | --- |
| 10 | 51 | M | 97.1 | 28.9 | 54 | --- | --- | --- |
| 11 | 46 | M | 80.3 | 24.7 | 71 | --- | --- | --- |
| 12 | 24 | M | 80.0 | 26.1 | 60 | --- | --- | --- |
| AVE | 39.75 | | 81.08 | 25.6 | 57 | --- | --- | --- |
| SD | 8 | | 16.25 | 2.9 | 9 | --- | --- | --- |
| p-value | 0.64 | --- | 0.72 | 0.84 | 0.22 | --- | --- | --- |

AB: Able-bodied; AIS: ASIA impairment scale where ASIA stands for American Spinal BMI: Body mass index; Injury Association; HR: Heart rate; SCI: Spinal cord injury; YPI: Years post injury

*Baseline Autonomic and Diastolic Data:*

All baseline autonomic data are provided in Table 2. There were no between-group differences in resting HR or any of the frequency and time domain measures of cardiac parasympathetic activity. In addition, there was no between-group difference in baseline QTVI values. Table 5 also shows that baseline diastolic parameters were not significantly different between the groups. Table 3 shows correlations between resting diastolic parameters and baseline indices of cardiac parasympathetic activity. Able-bodied individuals demonstrated strong correlations between E and various indices of cardiac vagal outflow, including QTVI, SDNN, RMSSD and CV, whereas E showed a strong trend for a correlation with HFln. The E:A ratio was also significantly correlated with RMSSD and showed a trend towards a correlation with QTVI. Individuals with SCI only demonstrated a negative correlation between DT and CV.

**Table 2: Baseline Cardiac Parasympathetic Indices**

| | AB | SCI | p-value |
|---|---|---|---|
| HR | 61 ± 13 | 62 ± 10 | 0.80 |
| HFln | 5.9 ± 1.4 | 6.2 ± 1.3 | 0.53 |
| SDNN | 55.30 ± 22.4 | 60.6 ± 23.1 | 0.56 |
| RMSSD | 42.1 ± 22.7 | 48.2 ± 27.1 | 0.55 |
| CV | 7.1 ± 2.2 | 6.4 ± 2.6 | 0.31 |
| QTVI | -1.4 ± 0.3 | -1.3 ± 0.7 | 0.72 |

AB: Able-bodiedSCI: Spinal cord injury; HR: heart rate; HFln: natural log of the high frequency power spectrum; SDNN: Standard deviation of continuous RR intervals; RMSSD: Root mean square of differences between RR intervals; CV: Coefficient of variance; QTVI: QT-variability index

**Table 3: Correlations between baseline cardiac parasympathetic activity and diastolic function.**

| | AB | SCI |
|---|---|---|
| | E | E |
| QTVI | r=-0.60; p=0.03* | r=-0.10; p=0.79 |
| HFln | r=0.54; p=0.06 | r=0.21; p=0.50 |
| SDNN | r=0.61; p=0.03* | r=0.18; p=0.51 |
| RMSSD | r=0.75; p=0.003* | r=0.28; p=0.36 |
| CV | r=-0.61; p=0.03* | r=0.12; p=0.69 |
| | A | A |
| QTVI | r=0.49; p=0.12 | r=0.41; p=0.21 |
| HFln | r=-0.02; p=0.96 | r=0.03; p=0.92 |
| SDNN | r=-1.55; p=0.63 | r=0.24; p=0.43 |
| RMSSD | r=-0.23; p=0.47 | r=0.08; p=0.79 |
| CV | r=0.17; p=0.59 | r=-0.21; p=0.48 |
| | E:A | E:A |
| QTVI | r=-0.54; p=0.06 | r=-0.33; p=0.33 |
| HFln | r=0.15; p=0.62 | r=0.13; p=0.66 |
| SDNN | r=0.36; p=0.23 | r=-0.05; p=0.86 |
| RMSSD | r=0.70; p=0.01* | r=0.11; p=0.72 |
| CV | r=-0.31; p=0.31 | r=0.31; p=0.31 |
| | DT | DT |
| QTVI | r=-0.15; p=0.63 | r=0.20; p=0.56 |
| HFln | r=0.10; p=0.75 | r=-0.19; p=0.53 |
| SDNN | r=0.18; p=0.55 | r=-0.28; p=0.35 |
| RMSSD | r=0.13; p=0.68 | r=-0.11; p=0.71 |
| CV | r=-0.31; p=0.34 | r=-0.60; p=0.04* |

*denotes significant correlation ($p \leq 0.05$); AB: Able-bodied; SCI: Spinal cord injury; QTVI: QT-variability index; HFln: Natural log of the high frequency power spectrum; SDNN: Standard deviation of continuous RR intervals; RMSSD: Root mean square of differences between RR intervals; CV: Coefficient of variance; E: Early transmitral velocity; A: Late transmitral velocity; E:A: Ratio of early to late transmitral velocity; DT: Deceleration time.

*Diastolic-Autonomic Function in Response to the CFT.*

All autonomic changes in response to the CFT are presented in Table 4. There was a significant group by condition (CFT) interaction for all the autonomic measures, demonstrating that the two groups responded differently to the CFT. The able-bodied

group demonstrated the typical autonomic responses to the CFT, which included a significant reduction in HR, and an increase in vagal cardiac outflow as shown by significant increases in HFln, SDNN, RMSSD, and CV. In contrast, the SCI group did not show any changes in HR or HFln, but did show a trend for increased SDNN, RMSSD and CV. In addition, the SCI group showed large effect sizes for the indices of cardiac parasympathetic indices. Furthermore, compared to the SCI group, the able-bodied group showed greater change in HR, HFln, SDNN, RMSSD and CV in response to the CFT.

**Table 4: Cardiac vagal response to the CFT**

| | Pre-CFT | CFT | p-Value | Cohen's' D | Change | p-value (for change) |
|---|---|---|---|---|---|---|
| **HR** | | | | | | |
| AB | 64 ± 13 | 56 ± 13* | 0.002 | 2.67 | -8 ± 8^ | 0.005 |
| SCI | 64 ± 11 | 65 ± 13 | 0.58 | 0.31 | 1 ± 8 | |
| **HFln** | | | | | | |
| AB | 5.9 ± 1.3 | 8.4 ± 2.2* | 0.0001 | 0.74 | 2.5 ± 1.8^ | 0.002 |
| SCI | 6.2 ± 1.3 | 6.8 ± 1.9 | 0.12 | 1.01 | 0.6 ± 1.4 | |
| **SDNN** | | | | | | |
| AB | 70.3 ± 30 | 244.6 ± 239* | 0.02 | 0.3 | 175 ± 232^ | 0.02 |
| SCI | 58.1 ± 24 | 85 ± 55 | 0.07 | 1.19 | 27.1 ± 49 | |
| **rMSSD** | | | | | | |
| AB | 47.8 ± 32.9 | 199.5 ± 217.1* | 0.02 | 0.42 | 152.7 ± 209.4^ | 0.03 |
| SCI | 46.7 ± 23.8 | 71.9 ± 59.8 | 0.07 | 1.19 | 25.2 ± 44.9 | |
| **CV** | | | | | | |
| AB | 7.4 ± 3.0 | 16.5 ± 1.0 * | 0.005 | 0.57 | 9.2 ± 9.4 ^ | 0.02 |
| SCI | 6.2 ± 2.7 | 8.7 ± 5.2 | 0.08 | 1.10 | 2.5 ± 4.8 | |

* denotes significantly different than Pre-CFT ($p \leq 0.05$); ^ denotes significantly different than SCI ($p \leq 0.05$); Pre-CFT: Pre cold face test; CFT: Cold face test; AB: Able-bodied; SCI: Spinal cord injury; HR: Heart rate; HFln: Natural log of high frequency; SDNN: Standard deviation of continuous RR intervals; RMSSD: Root mean square of differences between RR interval; CV: Coefficient of variance.

All LV diastolic data in response to the CFT are presented in Table 5. There were no group by condition interactions in any of the parameters, and no changes to any mean diastolic values in response to the CFT in both groups.

**Table 5: Left Ventricular Diastolic Function in response to the CFT.**

| | Pre-CFT | CFT | p-value (Interaction) | Change | p-value (for change) |
|---|---|---|---|---|---|
| **E** | | | | | |
| AB | 0.74 ± 0.1 | 0.71 ± 0.1 | 0.51 | -0.02 ± 0.1 | 0.41 |
| SCI | 0.75 ± 0.1 | 0.70 ± 0.1 | | -0.05 ± 0.09 | |
| **A** | | | | | |
| AB | 0.38 ± 0.1 | 0.41 ± 0.1 | 0.76 | 0.03 ± 0.1 | 0.54 |
| SCI | 0.44 ± 0.1 | 0.44 ± 0.1 | | 0.00 ± 0.1 | |
| **E:A** | | | | | |
| AB | 2.1 ± 0.6 | 1.8 ± 0.6 | 0.72 | -0.2 ± 0.7 | 0.81 |
| SCI | 1.8 ± 0.6 | 1.7 ± 0.5 | | -0.1 ± 0.4 | |
| **DT** | | | | | |
| AB | 203.4 ± 66.2 | 208.8 ± 72.1 | 0.56 | 5.4 ± 51.1 | 0.56 |
| SCI | 199.8 ± 41.6 | 218.5 ± 47 | | 18.7 ± 58.7 | |

AB: Able-bodied; SCI: Spinal cord injury; E: Early transmitral velocity; A: Late transmitral velocity; E:A: Ratio of early to late transmitral velocity; DT: Deceleration time.

Table 6 shows correlations between the changes in cardiac parasympathetic indices and the changes in diastolic parameters during the CFT. The able-bodied group showed significant correlations between changes in cardiac parasympathetic outflow and changes in early diastolic filling during the CFT. In particular, the change in E was positively correlated with the change in SDNN ($r=0.61$; $p=0.02$) as well as the change in HFln ($r=0.60$; $p=0.05$). After adjusting for change in HR, there was a trend between the change in E and change in SDNN ($r=0.54$; $p=0.09$), while there was no correlation between the change in E and change in HFln ($r=0.18$; $p=0.61$). In addition, the able-bodied participants showed a positive correlation between the change in E:A ratio and the change in CV ($r=0.61$; $p=0.04$), which was strengthened after adjusting for the change in

HR (r=0.72; p=0.04). Interestingly, the SCI group showed a trend for a negative correlation between the change in E and the change in HFln (r=-0.52; p=0.08). In addition, the SCI group also showed significant positive correlations between change in DT and change in HFln (r=0.7; p=0.01), as well as RMSSD (r=0.7; p=0.01), both of which showed strong correlations after adjusting for the change in HR (r=0.54; p=0.07 for HFln and RMSSD).

**Table 6: Correlations between change in cardiac vagal activity and change in diastole during the CFT**

| | **AB** | **SCI** |
|---|---|---|
| | △E | △E |
| △HFln | r=0.60; p=0.05* | r=-0.52; p=0.08 |
| △SDNN | r=0.61; p=0.02* | r=-0.10; p=0.97 |
| △RMSSD | r=0.46; p=0.14 | r=-0.13; p=0.66 |
| △CV | r=0.30; p=0.37 | r=0.01; p=0.97 |
| | △A | △A |
| △HFln | r=0.20; p=0.54 | r=-0.53; p=0.06 |
| △SDNN | r=-0.02; p=0.95 | r=-0.18; p=0.55 |
| △RMSSD | r=-0.03; p=0.92 | r=-0.18; p=0.55 |
| △CV | r=-0.25; p=0.47 | r=-0.26; p=0.40 |
| | △E:A | △E:A |
| △HFln | r=0.16; p=0.62 | r=0.02; p=0.95 |
| △SDNN | r=0.43; p=0.16 | r=0.11; p=0.73 |
| △RMSSD | r=0.42; p=0.17 | r=-0.02; p=0.94 |
| △CV | r=0.61; p=0.04* | r=0.22; p=0.47 |
| | △DT | △DT |
| △HFln | r=-0.19; p=0.56 | r=0.67; p=0.01* |
| △SDNN | r=-0.15; p=0.65 | r=0.52; p=0.12 |
| △RMSSD | r=-0.09; p=0.77 | r=0.68; p=0.01* |
| △CV | r=0.01; p=0.97 | r= 0.28; p=0.35 |

*denotes significant correlation (p≤0.05)AB: Able-bodied; SCI: Spinal cord injury; △: Change; E: Early transmitral velocity; HFln: natural log of the high frequency spectrum; SDNN: Standard deviation of continuous RR intervals; E:A: Ratio of early to late transmitral velocity; CV: Coefficient of variance; DT: Deceleration time RMSSD: Root mean square of differences between RR interval.

**Discussion:**

The main findings of the current investigation are that 1) able-bodied individuals who have greater parasympathetic outflow (as shown by HRV and QTVI) have greater early ventricular filling velocities, however this correlation is absent in individuals with SCI, 2) individuals with SCI demonstrate a blunted HR response to the CFT, despite strong trends towards increases in HRV and 3) cardiac vagal stimulation via the CFT is associated with improved LV filling in able-bodied individuals, yet reduced diastolic function in individuals with SCI. Together, these findings suggest an atypical disconnect between cardiac vagal outflow and both diastolic function and HR in individuals with SCI.

*Physiology of Cardiac Vagal-Diastolic Interaction:*

The isolated effect of parasympathetic activity on LV diastolic function is not very well understood. Part of the difficulty in discerning the isolated influence of autonomic activity on diastolic function is due to the concomitant effects of autonomic activity on HR, hemodynamic load and cardiac electrophysiology, all of which influence LV filling. Previous animal studies however suggest that vagal activity affects LV diastole, not by altering passive diastolic parameters (isovolumetric relaxation and/or LV compliance), but indirectly via left atrial function. For example, a previous study showed that direct vagal nerve stimulation in dogs increased LV filling as a result of an increase in left atrial pressure and left atrial-ventricular pressure gradient[16]. This is in agreement with work by Sarnoff et al., (1960), who also demonstrated an increase in left atrial pressure following direct vagus nerve stimulation in dogs[30]. In addition, vagal stimulation may also influence LV filling by reducing left atrial contractility, or late diastole.

Specifically, Williams et al. (1965) showed a 19% reduction in atrial contractility following direct vagus nerve stimulation in healthy dogs[8]. Conversely, studies in both animals and humans have shown that vagal withdrawal decreases early LV filling, partly by increasing atrial contractility[7,10]. Accordingly, vagal stimulation likely increases early diastolic filling by reducing left atrial contractility and increasing left atrial pressure[10,16].

*Baseline Autonomic-Diastolic Interactions:*

Regarding the first major finding of the study, at baseline, the able-bodied group demonstrated a positive correlation between early diastolic filling and cardiac parasympathetic activity, as shown by the QTVI and time and frequency measures of HRV. This is in agreement with results from Antelmi et al., (2010) who reported that healthy individuals with lower E:A ratios demonstrate lower HRV values[15]. Similarly, patients with diabetes who have lower HRV also demonstrate lower values of diastolic function[12]. This relationship however was not maintained in the present SCI group, as these individuals showed no correlations between early diastolic filling and cardiac parasympathetic activity. This suggests that there may be a disconnect between cardiac vagal activity and LV filling after SCI. Although it is unclear as to why the relationship between resting cardiac vagal activity and LV filling is lost in those with SCI, a possible explanation could be reduced baroreceptor sensitivity. Baroreceptor function was not assessed in the current study, however it has been shown to be reduced in individuals with SCI[31,32]. Baroreceptors in the left atrium modulate cardiac autonomic activity according to atrial pressure (which is dependent on left atrial volume). An increase in atrial pressure decreases sympathetic outflow and increases parasympathetic activity[33]. Accordingly, this increase in parasympathetic activity could augment diastolic filling[8,16].

Therefore, if the current SCI participants had impaired baroreceptor sensitivity, this may be responsible for the observed disconnect between cardiac parasympathetic activity and ventricular filling, however, further research is warranted in order to investigate the relationship between baroreceptor function and diastolic function in individuals with SCI.

*Autonomic & Diastolic Responses to the CFT:*

The CFT was used in this study as a non-invasive analogue for cardiac vagal stimulation[20,21]. Normally, facial surface nociceptors are stimulated when subjected to a cold stimulus, and this activates the maxillary and opthalamic branches of the trigeminal nerves which are mainly sensory in function. Afferent nociceptor signals from the trigeminal nerve increase vagal efferent signals, which result in pronounced bradycardia[34]. Regarding the second major finding of the study, the able-bodied group demonstrated the typical physiological response to the CFT, which included an elevation in cardiac parasympathetic activity, as shown by elevation in HRV, and a significant reduction in HR. This has been repeatedly shown before in healthy humans suggesting intact cardiac vagal function[20,21,35]. In addition, since breath holding or changing breathing rate during the CFT can augment the resultant bradycardia[36,37], participants in the current study were continuously instructed to maintain their normal breathing rate during the test. Therefore, the observed changes in HRV and HR were likely due to alterations in vagal activity and not changes in breathing. In contrast, the SCI group exhibited a strong trend towards an increase in cardiac vagal activity (increased HRV), however, they did not show changes in HR during the CFT. It is important to note the effect sizes for HRV parameters were quite large, suggesting that the SCI did exhibit an increase in HRV, but statistical significance was not reached due to the relatively small

sample size. Blunted or reduced bradycardic response to facial cooling has been previously documented in older individuals[38,39], suggesting that individuals with SCI may exhibit accelerated cardiovascular aging. Although such accelerated cardiovascular aging has been suggested to be partly due to the profound physical inactivity following SCI[19,40], this is likely not the case here, as the majority of the participants in this study participated in mild weekly exercise and used manual wheelchairs for transportation. The only other study to employ the CFT in individuals with SCI reported no changes in cardiac vagal activity and an unexpected tachycardia[19]. This inconsistency in results is interesting especially that the participants involved in the study by Wecht et al (2009) were similar to our participants with respect to age, years post injury and level of injury. A possible explanation for the difference in results could be from the difference in facial cooling temperatures used in the studies. Wecht et al. (2009) performed the CFT at a temperature of 4◦C, whereas we did it at a temperature of 1◦C. Heath et al (1990) showed that colder temperatures during the CFT elicit greater parasympathetic responses than warmer temperatures[41], and therefore face cooling at a temperature of 4C may have not been sufficient enough to elicit a vagal response, or an increase in HRV in those with SCI. However, regardless of a blunted[19] or augmented vagal response (current study) to the CFT, individuals with SCI still show atypical HR activity to the CFT. Such paradoxical HR responses to facial cooling has been hypothesized to be due to possible alterations in cardiac cholinergic receptors[19], however, further investigations are warranted. In addition, potential impairments to baroreceptor sensitivity likely did not contribute to the blunted HR response in the SCI group, as the CFT has been shown to operate independently from baroreceptor function[20,21].

Regarding the third major finding of the study, the CFT was also used to increase vagal stimulation in order to further understand diastolic-autonomic dynamics. During the CFT, the able-bodied group showed positive correlations between increases in HRV and diastolic filling (E and E:A ratio). This relationship was maintained after controlling for changes in HR, suggesting that the increased ventricular filling was not entirely driven by the change in HR, but by parasympathetic activity. Although atrial pressure was not measured in the current study, we speculate that an increase in atrial pressure may have resulted in the augmented early diastolic filling in the able-bodied individuals[16,30]. This diastolic response to elevated cardiac vagal activity has been previously demonstrated in experimental models[8,16], suggesting that the able-bodied group displayed normal autonomic-diastolic interactions. Interestingly enough, the SCI group displayed an atypical diastolic response to the increased HRV during the CFT. Specifically, there was a trend for a negative association between the change in E and the change in HFln during the CFT. In addition, the increase in HRV was strongly associated with prolongation of DT in individuals with SCI. These correlations suggest that unlike the able-bodied group, the SCI group exhibit a paradoxical reduction in left ventricular filling efficiency in response to elevated cardiac parasympathetic activity. This does not necessarily mean that vagal activity is detrimental to cardiac function after SCI, but it may be pointing at an underappreciated alteration in autonomic modulation of cardiac function, which requires further investigation.

As such, this data presents novel evidence for atypical HR and diastolic responses to cardiac vagal activity and a potential disconnect between autonomic and cardiac function in individuals with SCI. It is difficult to discern the source or mechanisms

behind such physiological alterations from the methods used in the current study. This is because ECG-based assessments of cardiac autonomic function are a downstream measure that reflects the collective actions of supraspinal activity, vagus nerve outflow, cholinergic receptor uptake of acetylcholine, and electric conduction throughout the heart. As such, the impairments that led to the observed autonomic-cardiac disconnect in the SCI group can be attributed to one or more of any of these steps. Therefore, further studies are warranted in order to elucidate the source of physiological dysfunction.

**Conclusion:**

Resting cardiac vagal outflow is associated with early LV filling in able-bodied individuals, however this relationship is lost in those with SCI. In addition, increasing cardiac vagal outflow via the CFT is associated with increasing early diastolic filling in able-bodied individuals, but is associated with impaired LV diastole in those with SCI. Furthermore, individuals with SCI demonstrate a blunted HR response to increased cardiac vagal activity during the CFT.

**References:**


1) Assa S, Hummel Y, Voors A: Changes in left ventricular diastolic function during hemodialysis sessions. Am J Kidney Dis 2013; 62:549-56.

2) Borlaug BA, Melenovsky V, Redfield MM et al: Impact of arterial load and loading sequence on left ventricular tissue velocities in humans. J Am Coll Cardiol 2007; 50:1570-77.

3) Carrick-Ranson G, Hastings JL, Bhella PS: Effect of healthy aging on left ventricular relaxation and diastolic suction. Am J Physiol Heart Circ, 2012; *303:*H315-H322.

4) Fujimoto N, Borlaug BA, Lewis GD, et al: Hemodynamic responses to rapid saline loading: the impact of age, sex, and heart failure. Circulation 2012; 55-62.

5) Gaasch W, Zile M: Left ventricular diastolic dysfunction and diastolic heart failure. Annu Rev Med 2004, 55:373-94.

6) Brubaker P, Kaminsky L, Whaley M: Coronary artery disease: essentials of prevention and rehabilitation programs. Muncie, Indiana: Human Kinetics. 2002. *Coronary artery disease: essentials of prevention and rehabilitation programs*. Muncie, Indiana: Human Kinetics.

7) Johannessen KA, Cerqueira M, Veith RC, Stratton JR: Influence of sympathetic stimulation and parasympathetic withdrawal on Doppler echocardiographic left ventricular diastolic filling velocities in young normal subjects. AM J Cardiol 1991; 67:520-6.

8) Williams JF, Sonnenblick EH, Braunwald E: Determinants of atrial contractile force in the intact heart. Am J Physiol Leg Cont 1965; 2*09:*1061-8.

9) Finkelhor RS, Karwa P, Kaufman ES, Bahler RC: The relationship of autonomic function to age-related changes in left ventricular filling in normal subjects. Eur Heart J 1995; 16:1716-20.

10) Stratton JR, Levy WC, Caldwell JH, et al: Effects of aging on cardiovascular responses to parasympathetic withdrawal. J Am Coll Cardiol 2003; 41:2077-83.

11) Poirier P, Bogaty P, Philippon F, et al: Preclinical diabetic cardiomyopathy: relation of left ventricular diastolic dysfunction to cardiac autonomic neuropathy

in men with uncomplicated well-controlled type 2 diabetes. Metabolism 2003; 52:1056-61.

12) Poanta L, Porojan M, Dumitrascu DL: Heart rate variability and diastolic dysfunction in patients with type 2 diabetes mellitus. Acta Diabetol 2011; 48:191-6.

13) Poulsen SH, Jensen SE, Møller JE, Egstrup K: Prognostic value of left ventricular diastolic function and association with heart rate variability after a first acute myocardial infarction. Heart 2001; 86: 376-80.

14) Ishise H, Asanoi H, Ishizaka S, et al: Time course of sympathovagal imbalance and left ventricular dysfunction in conscious dogs with heart failure. J Appl Phys 1998; 84:1234-41.

15) Antelmi I, Yamada A T, Hsin CN, et al: Influence of parasympathetic modulation in Doppler mitral inflow velocity in individuals without heart disease. J Am Soc Echocardiog 2010; 23:762-65.

16) Xenopoulos NP, Applegate RJ: The effect of vagal stimulation on left ventricular systolic and diastolic performance. Am J Physiol Heart Circ Physiol 1994; 266: H2167-H2173.

17) Teasell RW, Arnold JM, Krassioukov A, Delaney GA: Cardiovascular consequences of loss of supraspinal sympathetic nervous system after spinal cord injury. Arch Phys Med Rehabil 2000; 81:506-16.

18) Wecht JP, Weir JP, Bauman WA. Blunted heart rate response to vagal withdrawal in persons with tetraplegia. Clin Auton Res 2006b; 16:378-83.

19) Wecht JM, Weir JP, DeMeersman RE, et al: Cold face test in persons with spinal cord injury: age versus inactivity. Clin Auton Res 2009, 19: 221-229.

20) Kinoshita T, Nagata S, Baba R, et al: Cold-water face immersion per se elicits cardiac parasympathetic activity. Circ J 2006; 70:773-6.

21) Khurana RK, Wu R: The cold face test: a non-baroreflex mediated test of cardiac vagal function. Clin Auton Res 2006; *16:*202-7.

22) Chen GY, Kuo CD: The effect of the lateral decubitus position on vagal tone. Anaesthesia 1997; 52:653-7.

23) Sharif H, Allison D, O'Leary D, Ditor D: Reproducibility of the QT-variability index in individuals with spinal cord injury. Auton Neurosci 2016; 195:16-9.

24) Sharif H, Cotie LM, La Fountaine MF, Ditor DS. The influence of cardiac autonomic activity on the QT-variability index in able-bodied and incomplete spinal cord injured individuals. Auton Neurosci *2015; 190:46-52*.

25) Berger RD, Kasper EK, Baughman KL: Beat-to-beat QT interval variability novel evidence for repolarization lability in ischemic and nonischemic dilated cardiomyopathy. Circulation 1997; 96:1557-65.

26) Nagueh SF, Appleton CP, Gillebert TC, et al: Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiog 2009; 22:107-33.

27) Shuitema K, Holm B: The role of differential areas in eliciting human diving bradycardia. Acta Physiol Scand 1988; 132:119-20.

28) Cohen J: Statistical power analysis for the behavioral sciences ($2^{nd}$ ed), NJ: Erlbaum. 281,284,285.

29) Sacha J: Why should one normalize heart rate variability with respect to average heart rate. Front Physiol 2013; 4:306.

30) Sarnoff SJ, Brockman SK, Gilmore JP, Linden RJ, Mitchell JH: Regulation of ventricular contraction. Influence of cardiac sympathetic and vagal nerve simulation on atrial and ventricular dynamics. Circ Res 1960; 8:1108-22.

31) Claydon VE, Krassioukov AV: Clinical correlates of frequency analyses of cardiovascular control after spinal cord injury. Am J Phys Heart Circ Physiol. 294:H668-H678.

32) Krum H, Lous W, Brown D, Howes L: Pressor dose responses and baroreflex sensitivity in quadriplegic spinal cord injury patients. J Hyperten 1992; 10:245-50.

33) Klabunde R: Cardiovascular Physiology Concepts. Trade Paperback 2011.

34) Gooden BA: Mechanisms of the human diving response. Integ Physiol Behav Sci 1994; 29:6-16.

35) Stemper B, Hilz MJ, Rauhut U, Neundorfer B: Evaluation of cold face test bradycardia by means of spectral analysis. Clin Auton Res 2002; 12:78-83.

36) Reyners AKL, Tio RA, Vlutters FG, et al: Re-evaluation of the cold face test in humans. Eur J Appl Physiol 2000; 82:487-92.

37) Kawakami Y, Natelson BH, DuBois AR: Cardiovascular effects of face immersion and factors affecting diving reflex in man. J Appl Physiol 1967; 23:964-70.

38) Collins KJ, Abdel-Rahman TA, Easton JC, et al: Effects of facial cooling on elderly and young subjects: interactions with breath-holding and lower body negative pressure. Clin Sci 1996; 90:485-92.

39) Petrella RJ, Cunningham DA, Nichol PM, Thayer R: Cardiovascular responses to facial cooling are age and fitness dependent. Med Sci Sports Exerc 1999; 31:1163-8.

40) De Groot PC, Van Kuppevelt DH, Pons C, et al: Time course of arterial vascular adaptations to inactivity and paralysis in humans. Med Sci Sports Exerc 2003; 35:1977-85.

41) Heath ME, Downey JA: The cold face test (diving reflex) in clinical autonomic assessment: methodological considerations and repeatability of responses. Clin Sci 1990; 78:139-47.

## Chapter 7

## Manuscript 4

# The Effect of Blood Volume and Volume Loading on Left Ventricular Diastolic Function in Individuals with Spinal Cord Injury

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**Abstract:**

**Background:** There remains a lack of consensus on alterations in diastolic function following spinal cord injury (SCI). Hypovolemia following paralysis may play a role in the putative diastolic impairments, however, diastolic function has only been examined during resting conditions in those with SCI.

**Objectives:** 1) To examine diastolic function at rest and during rapid saline infusion in those with SCI and 2) to determine the contribution of blood volume to the purported diastolic impairments in SCI.

**Methods:** Thirteen SCI (Age: 41±8.5; 10 males, 3 females) and 12 able-boded (AB) individuals (Age: 40±8.5; 9males, 3 females) without history of cardiovascular disease participated in the study. M-Mode and 2-dimensional echocardiography were used to assess left ventricular structure as well as pulse-wave Doppler and tissue Doppler indices of diastole. The carbon monoxide rebreathe test was employed to measure blood volume. Rapid saline infusion was performed to increase preload (Dose:15mL/Kg; rate:100mL/min) and diastolic function was assessed once more after volume loading.

**Results:** Individuals with SCI had smaller LV internal diameter (SCI: 4.5±0.3 cm vs AB: 5.1±0.7cm; p=0.01) and lower preload, as evidenced by lower blood volume (SCI: 3.9L±0.6L vs AB: 5.0±1.2L; p=0.02) and end diastolic volume (SCI: 97.2±29.4ml vs AB: 128.6±38.3ml; p=0.03). There were no differences in any of the baseline diastolic velocity parameters between groups, however when LV internal diameter was adjusted for, individuals with SCI demonstrated lower early to late transmitral velocity ratio (SCI: 1.9±0.5 vs AB: 2.2±0.7; p=0.03). There were no between-group differences in diastolic responses to the saline infusion, as both groups showed similar degrees of diastolic velocity augmentations following volume loading.

**Conclusion:** Individuals with SCI have preserved diastolic function despite having lower preload. Cardiac atrophy in SCI may serve as a positive adaptation that maintains normal diastolic velocities. Individuals with SCI also demonstrate similar diastolic responses to increased preload in comparison to an able-bodied control group, suggesting compliant ventricles.

**Key Words:** Spinal cord injury, diastolic function, saline infusion, blood volume, preload

**Introduction:**

Individuals with spinal cord injury (SCI) represent one of the most physically inactive populations, due to their profound immobility. Accordingly, such immobility results in an elevated risk of a wide array of cardiovascular complications, including coronary artery disease[1], peripheral artery disease[2], atherosclerosis[3] and accelerated cardiovascular aging[4]. Although severe physical inactivity has been shown to be associated with impaired diastolic function in able-bodied individuals[5-7], findings regarding diastolic function after SCI remain equivocal. Left ventricular diastolic function has been shown by some to be preserved in those with SCI[8-10] while more recent reports convey diastolic impairments after SCI[11-14]. Therefore, it is unknown if diastolic dysfunction actually manifests after spinal cord injury.

It is important to note that echocardiographic parameters of diastolic function are highly influenced by preload. Specifically, an increase in preload augments diastolic values while a decrease in preload reduces them[15-18]. Individuals with SCI demonstrate reduced ventricular filling and preload, partly due to a reduction in blood volume secondary to chronic physical inactivity[19]. Therefore, it is not clear if the reported diastolic impairments following SCI are a result of reduced blood volume or due to pathological alterations in the left ventricle, *per se*. In addition, diastolic function in individuals with SCI have previously only been examined while participants were in a relaxed state, and therefore it is unclear how ventricular filling after SCI functions when the heart is exposed to stress. Rapid saline infusion is a diagnostic method that increases preload, and may reveal left ventricular impairments that may otherwise not manifest during resting conditions[20]. In addition, the normal diastolic responses to rapid saline

infusion are well documented[17,18], and therefore this method maybe a useful tool to elucidate any potential left ventricular diastolic impairments in individuals with SCI.

Accordingly, the purpose of this study was to determine if individuals with SCI demonstrate diastolic impairments at rest and/or during a volume stress induced by rapid saline infusion. We also aimed to determine if a reduction in blood volume following SCI contributes to the putative diastolic impairments in this population.

**Methods:**

*Participants:*

Thirteen individuals with spinal cord injury (C4-T5; 7 complete, 6 incomplete; 16.5±9.7 years post injury) and 12 able-bodied individuals matched for age, sex and body mass participated in this study. Participants were non-smokers, non-hypertensive, did not have a history of heart disease and had normal resting and exercise electrocardiograms. As this is the first study to perform rapid saline infusion in individuals with SCI, those with unstable autonomic dysreflexia were excluded. Participants from both groups included a range of sedentary to physically active individuals but none were competitive or highly trained athletes. All participants provided informed consent to participate in the study, which was approved by the Brock University Biosciences Research Ethics Board.

*General Study Protocol:*

All participants were required to void their bladder before testing, especially those with SCI in order to reduce any risk of autonomic dysreflexia. Body mass (kg) and height were measured and from this body mass index (BMI: $kg/m^2$) was calculated. After a 15-minute resting period in the left lateral decubitus position, resting blood pressure was obtained manually from the brachial artery and a continuous electrocardiogram recording

(lead II) was taken throughout the testing session. Following the rest period, assessment of left ventricular structure and function was performed via 2-Dimensional and M-mode echocardiography. Participants then performed the carbon monoxide rebreathe test for the measurement of total blood volume. This was then followed by rapid saline infusion. After 5 minutes of saline infusion, left ventricular diastolic function was assessed once more.

*Echocardiography:*

All echocardiographic images were obtained using a commercially available ultrasound system (Vivid Q; GE Vingmed Ultrasound AS, Horten, Norway) with a 1.5-MHz phased-array transducer. All images were acquired by a single experienced sonographer and were stored for later offline analysis using commercially available software (EchoPac version 6.0; GE Vingmed Ultrasound AS). Images were obtained while participants were in the left lateral decubitus position at end expiration and 3 consecutive cycles for each image were stored and averaged for offline analysis.

Left ventricular wall dimensions were assessed at end diastole and end systole via M-mode recordings (and values were confirmed by 2-dimensional recordings) in accordance the recommendations of the American Society of Echocardiography (ASE) (21). Left ventricular wall dimensions included intraventricular septal thickness at diastole (IVSd) and systole (IVSs), posterior left ventricular internal diameter at diastole (LVIDd) and systole (LVIDs) as well as posterior wall thickness in diastole (PWTd) and systole (PWTs). Hemodynamic measures included end-diastolic volume, end-systolic volume, ejection fraction, as well as stroke volume. These measures were taken from the long parasternal axis view through M-mode, as apical chamber views from individuals

with SCI are suboptimal for the use of Simpson's Biplane. Cardiac output was calculated as the product of stroke volume and heart rate. Left ventricular mass (LVM) was calculated using the Devereux formula[22] and was indexed for body surface area (LVMi)[23].

Pulsed-wave Doppler and tissue Doppler were employed to assess diastolic function according to the recommendation of the American Society of Echocardiography[24]. For mitral inflow velocity, from an apical 4-chamber view, a 4mm sample volume was placed between the mitral valve leaflet tips during diastole. The Doppler velocity curves were digitized to obtain peak early (E) and late (A) mitral flow velocities, and their ratio (E:A) was calculated. The descending limb of the E wave was used to measure deceleration time (DT) and isovolumetric relaxation time (IVRT) was measured as the time between aortic valve closure and mitral valve opening. Mitral annular velocities were measured via tissue-Doppler imaging from the 4-chamber apical view. A 2mm sample volume was placed along the longitudinal movement of the basal septal and basal lateral walls. Peak early (E') and late (A') diastolic myocardial tissue velocities were recorded and E:E' was calculated as a non-invasive measure of left ventricular filling pressure[25].

*Carbon-Monoxide Rebreathe Test:*

The carbon-monoxide rebreathe test was employed for the assessment of total blood volume in accordance to the Burge and Skinner method[26]. Briefly, a 20-gauge catheter was placed in the antecubital vein for instant blood draws. A baseline blood sample was taken for measurement of hematocrit. After a 4-minute period of breathing 100% oxygen (Praxair), participants inhaled a 15-20ml priming dose of 100% CO gas

(Praxair) and a 1mL blood sample was obtained in a heparinized vacutainer for assessment of carboxy-hemoglobin and total-hemoglobin. A second dose of carbon-monoxide gas (40-50ml) was inhaled, and after 10 minutes of breathing, a final blood sample was obtained for carboxy-hemoglobin assessment. All blood samples were analyzed by a blood-gas analyzer via photospectromery (Phox-Cooximeter; NovaBiomedical). The carbon-monoxide rebreathe test yields a low reproducibility error in both able-bodied[27] and SCI individuals[19] and total blood volume was reported in absolute values and indexed for body mass.

*Volume Loading:*

Preload was increased via rapid infusion of warm isotonic saline (0.9% NaCl) through the antecubital vein by a 18-20 gauge catheter. The saline dose consisted of 15mL/kg at a rate of 100mL/min.

*Statistical Analysis:*

A Shapiro's Wilkis test was used to check for normalcy in all parameters, and as a result, parametric statistics were justified. Unpaired t-tests were used to compare all baseline left ventricular structural, diastolic and hemodynamic parameters between groups. An analysis of covariance (ANCOVA) was used to assess differences in baseline diastolic parameters while adjusting for LVIDd. The LVIDd was used as a covariate because a reduction in left ventricular size following SCI has been proposed as a mechanism to maintain normal diastolic function despite a reduction in preload[8,28] and LVIDd had an equal regression coefficient associated with both groups (assumption of homogeneity of regression). A 2-way repeated measures analysis of variance (ANOVA) was used to detect

group by condition (IV infusion) interactions as well as main effects for diastolic parameters. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS 22) software. Data were reported as means ± standard deviation, and level of statistical significance was set at $p \leq 0.05$.

**Results:**

*Participants:*

All participant characteristics are provided in Table 1. There were no between group differences in age, body mass, BMI or heart rate ($p > 0.05$). All participants tolerated the rapid saline infusion well and no adverse events occurred.

**Table 1: Participant characteristics**

| Participant | Age (years) | Sex | Body Mass(kg) | BMI(kg/m$^2$) | HR(bpm) | Level | AIS | YPI |
|---|---|---|---|---|---|---|---|---|
| **SCI** | | | | | | | | |
| 1 | 55 | M | 80.7 | 26.8 | 73 | C4 | A | 8 |
| 2 | 42 | M | 68.2 | 20.8 | 53 | C4 | C | 18 |
| 3 | 31 | F | 64.0 | 22.7 | 61 | C5 | B | 6 |
| 4 | 46 | M | 83.0 | 28.7 | 65 | T6 | A | 19 |
| 5 | 23 | M | 83.2 | 27.8 | 57 | C8 | B | 5 |
| 6 | 43 | M | 81.0 | 27.1 | 43 | C4 | A | 6 |
| 7 | 46 | F | 61.0 | 22.7 | 73 | C7 | A | 9 |
| 8 | 37 | M | 100.0 | 31.6 | 70 | C5 | B | 18 |
| 9 | 38 | M | 96.4 | 25.9 | 54 | C5 | C | 38 |
| 10 | 54 | M | 95.0 | 29.3 | 55 | C567 | D | 27 |
| 11 | 38 | F | 56.0 | 21.1 | 64 | T3 | A | 20 |
| 12 | 41 | M | 66.0 | 24.2 | 77 | T5 | A | 16 |
| 13 | 44 | M | 91.0 | 27.2 | 67 | T4 | A | 24 |
| AVE | 41.4 | | 78.9 | 25.8 | 62 | --- | --- | 16.5 |
| SD | 8.6 | | 14.6 | 3.3 | 9 | --- | --- | 9.7 |
| **AB** | | | | | | | | |
| 1 | 44 | M | 94.3 | 26.3 | 50 | --- | --- | --- |
| 2 | 44 | M | 70.1 | 24.8 | 47 | --- | --- | --- |
| 3 | 35 | F | 57.2 | 21.2 | 75 | --- | --- | --- |
| 4 | 35 | M | 76.0 | 24.5 | 62 | --- | --- | --- |
| 5 | 41 | M | 102.0 | 30.5 | 51 | --- | --- | --- |
| 6 | 44 | F | 68.3 | 24.9 | 43 | --- | --- | --- |
| 7 | 30 | F | 57.4 | 20.9 | 63 | --- | --- | --- |
| 8 | 33 | M | 91.1 | 27.8 | 51 | --- | --- | --- |
| 9 | 54 | M | 102.1 | 27.4 | 62 | --- | --- | --- |
| 10 | 51 | M | 97.1 | 28.9 | 54 | --- | --- | --- |
| 11 | 46 | M | 80.3 | 24.6 | 71 | --- | --- | --- |
| 12 | 24 | M | 80.0 | 26.1 | 60 | --- | --- | --- |
| AVE | 40.1 | | 81.2 | 25.7 | 57 | --- | --- | --- |
| SD | 8 | | 16.2 | 2.8 | 9 | --- | --- | --- |
| p-value | 0.71 | --- | 0.71 | 0.90 | 0.22 | --- | --- | --- |

AB: Able-bodied; AIS: ASIA impairment scale where ASIA stands for American Spinal BMI: Body mass index; Injury Association; HR: Heart rate; SCI: Spinal cord injury; YPI: Years post injury

*Baseline Left Ventricular Structure and Hemodynamics:*

Compared to able-bodied participants, individuals with spinal cord injury had significantly smaller LVIDd, and lower end diastolic volume, stroke volume and cardiac output, however ejection fraction was not different between groups (Table 2). There were no between-group differences in LVM, LVMi or any of the other structural parameters. Absolute total blood volume and blood volume indexed for body mass were significantly lower in the spinal cord injury group (Table 2). In addition, there were no between-group differences in any of the diastolic parameters (Table 3), however after controlling for LVIDd, the spinal cord injury group demonstrated a significantly lower E:A ratio (Table 3).

**Table 2: Baseline LV structural and hemodynamic parameters**

| | AB | SCI | P-value |
|---|---|---|---|
| **IVSd(cm)** | 1.0 ± 0.2 | 1.1 ± 0.3 | 0.30 |
| **LVIDd(cm)** | 5.1 ± 0.7* | 4.5 ± 0.5 | 0.01 |
| **LVPWd(cm)** | 1.1 ± 0.3 | 1.0 ± 0.2 | 0.61 |
| **IVSs(cm)** | 1.7 ± 0.7 | 1.4 ± 0.4 | 0.20 |
| **LVIDs(cm)** | 3.2 ± 0.3 | 2.9 ± 0.5 | 0.22 |
| **LVPWs(cm)** | 1.7 ± 0.5 | 1.5 ± 0.3 | 0.17 |
| **LVM(g)** | 201.9 ± 94 | 164.4 ± 51.6 | 0.24 |
| **LVMi($g/m^2$)** | 104.6 ± 46.1 | 87.3 ± 23.0 | 0.24 |
| **EDV(ml)** | 128.6 ± 38.3* | 97.2 ± 29.4 | 0.03 |
| **ESV(ml)** | 39.2 ± 11.6 | 32.75 ± 12.2 | 0.31 |
| **EF(%)** | 68.7 ± 6.2 | 64.5 ± 6.1 | 0.10 |
| **SV(ml)** | 88.8 ± 31* | 61.9 ± 18.2 | 0.01 |
| **CO (L/min)** | 4.9 ± 1.6* | 3.6 ± 0.89 | 0.02 |
| **Blood Volume (L)** | 5.0 ± 1.2* | 3.9 ± 0.6 | 0.02 |
| **Blood Volume (mL/Kg)** | 65.4 ± 10.5* | 52.7 ± 11.4 | 0.01 |

* denotes significantly different from SCI group ($p<0.05$); AB: Able-bodied; SCI: spinal cord injury; IVSd: Intraventricular septal wall thickness in diastole; LVIDd: Left ventricular internal diameter in diastole; LVPWd: Left ventricular posterior wall in diastole; IVSs: Intraventricular septal wall thickness in systole; LVIDs: Left ventricular internal diameter in systole; LVPWs: Left ventricular posterior wall thickness in systole; LVM: Left ventricular mass; LVMi: Left ventricular mass indexed for body surface area; EDV: End diastolic volume; ESV: End systolic volume; EF: Ejection fraction; SV: Stroke volume; CO: Cardiac output.

**Table 3: Baseline diastolic function before and after controlling for LV structure**

| | AB | SCI | P-Value | P-Value (with LVIDd as a covariate) |
|---|---|---|---|---|
| **E (m/sec)** | 0.77 ± 0.12 | 0.76 ± 0.11 | 0.39 | 0.64 |
| **A (m/sec)** | 0.38 ± 0.11 | 0.42 ± 0.08 | 0.26 | 0.09 |
| **E:A** | 2.16 ± 0.72* | 1.9 ± 0.51 | 0.14 | 0.03 |
| **DT (ms)** | 197.8 ± 61.0 | 210.7 ± 48.4 | 0.81 | 0.92 |
| **IVRT (ms)** | 103.6 ± 18.4 | 102.8 ± 18.8 | 0.82 | 0.43 |
| **E'Sept** | 0.12 ± 0.03 | 0.13 ± 0.02 | 0.84 | 0.70 |
| **E'Lat** | 0.14 ± 0.03 | 0.16 ± 0.04 | 0.22 | 0.30 |
| **A'Sept** | 0.08 ± 0.02 | 0.10 ± 0.03 | 0.19 | 0.11 |
| **A'Lat** | 0.08 ± 0.03 | 0.08 ± 0.03 | 0.79 | 0.33 |
| **EE'** | 6.1 ± 1.4 | 5.4 ± 1.07 | 0.10 | 0.96 |

* denotes significantly different from SCI group after controlling for LVIDd ($p<0.05$); AB: Able-bodied; SCI: Spinal cord injury; E: Early transmitral diastolic velocity; A: Late transmitral diastolic velocity; E:A: Early to late diastolic velocity ratio; DT: Deceleration time; IVRT: Isovolumetric relaxation time; E'Sept; Early septal annular myocardial diastolic velocity; E'Lat: Early lateral annular diastolic velocity; A'Sept: Late septal annular myocardial diastolic velocity; A'Lat: Late lateral myocardial diastolic velocity; EE': Ventricular filling pressure.

*Diastolic Response to Rapid Saline Infusion:*

There was no difference in the average amount of saline given to both groups (SCI:1184±214mL vs able-bodied: 1208±252mL; $p=0.80$). There were no group by condition interactions for any of the diastolic measures ($p>0.05$), suggesting that both groups responded similarly to the rapid saline infusion. However, there was a significant main effect for condition, specifically when collapsing across groups, the saline infusion resulted in a significant increase in E (0.78 ± 0.10m/sec to 0.90 ± 0.11m/sec; $p<0.01$; Figure 1a) and the E:A ratio (2.06 ± 0.61 to 2.82 ± 1.05; $p<001$; Figure 1b), as well as a significant decrease in A (0.40 ± 0.09m/sec to 0.35 ± 0.11m/sec; $p=0.05$; Figure 1c), DT (208.37 ± 50.04ms to 183.16 ± 37.21ms; $p=0.01$; Figure 1d) and IVRT (102.04 ± 17.54ms to 88.42

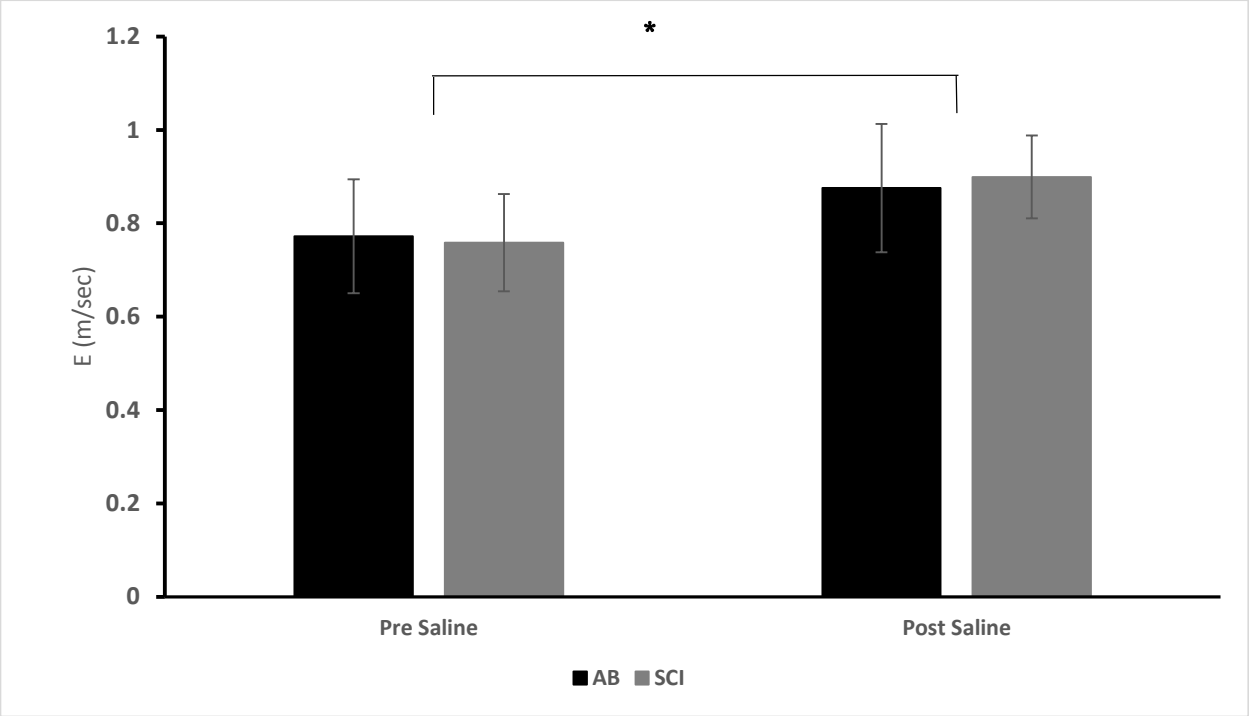
± 14.11ms; p<0.01; Figure 1e). The saline infusion also resulted in a significant increase in E'Sept (0.13 ± 0.02m/sec to 0.14 ± 0.03 m/sec; p=0.01) and E'Lat (0.15 ± 0.04m/sec to 0.17 ± 0.04m/sec; p<0.001) velocities. Rapid saline infusion resulted in no changes in A'Sept velocity (0.09 ± 0.02m/sec to 0.10 ± 0.03; p=0.20), A'Lat velocity (0.08 ± 0.03m/sec to 0.08 ± 0.03m/sec; p=0.40) or left ventricular filling pressure as indicated by E:E' (5.8 ± 1.3 to 6.0 ± 1.2; p=0.40). In addition, Table 4 shows that the percent change in diastolic values in response to the saline infusion was similar between groups.

**Table 4: Percent change in diastolic function after rapid saline infusion**

| | AB | SCI | P-Value |
|---|---|---|---|
| **E** | 14.1 ± 21.0 | 19.9 ± 15.9 | 0.43 |
| **A** | -4.8 ± 33.2 | -12.9 ± 33.2 | 0.49 |
| **EA** | 32.3 ± 47.6 | 47.1 ± 42.2 | 0.42 |
| **DT** | -7.5 ± 21.8 | -11.7 ± 17.3 | 0.6 |
| **IVRT** | -11.8 ± 20.6 | -13.8 ± 13.1 | 0.78 |
| **E'Sept** | 13.9 ± 18.5 | 13.5 ± 26.0 | 0.96 |
| **E'Lat** | 17.5 ± 20.5 | 7.9 ± 16.4 | 0.22 |
| **A'Sept** | 13.1 ± 22.7 | 2.3 ± 19.8 | 0.22 |
| **A'Lat** | 2.4 ± 16.6 | -10.7 ± 19.2 | 0.09 |
| **EE'** | 1.4 ± 24.3 | 0.3 ± 17.1 | 0.28 |

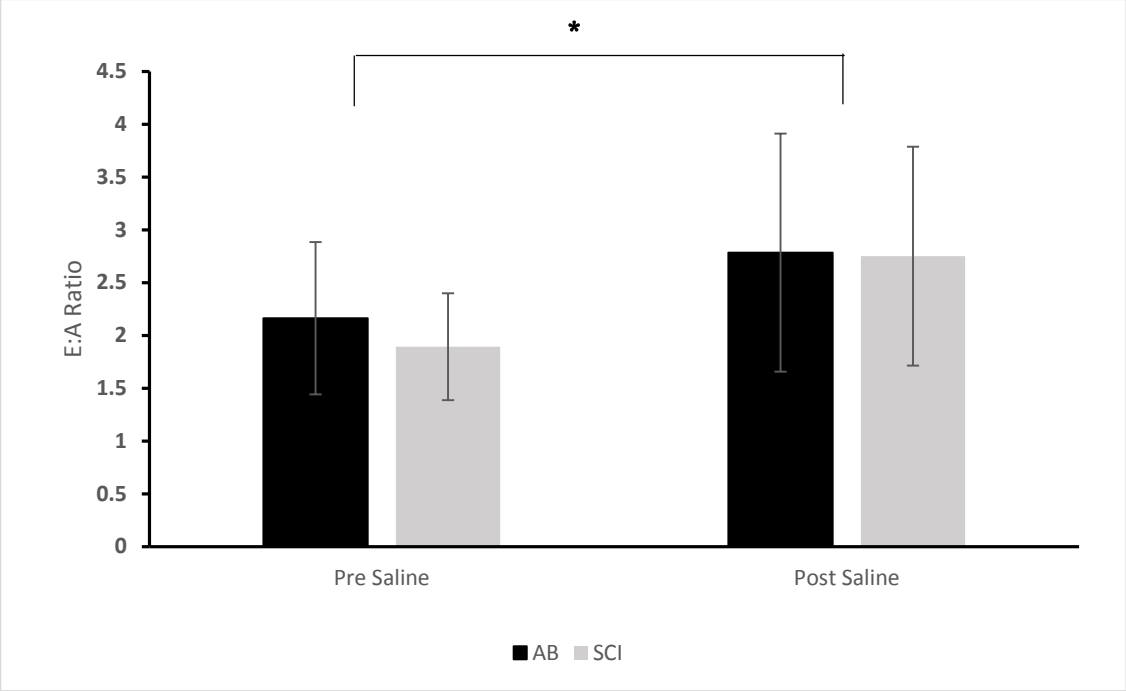
AB: able-bodied; SCI: spinal cord injury; E: early transmitral diastolic velocity; A: late transmitral diastolic velocity; E:A: early to late diastolic velocity ratio; DT: deceleration time; IVRT: isovolumetric relaxation time; E'Sept; early septal annular myocardial diastolic velocity; E'Lat: early lateral annular diastolic velocity; A'Sept: late septal annular myocardial diastolic velocity; A'Lat: late lateral myocardial diastolic velocity; EE': ventricular filling pressure.

**Figure 1a: Early transmitral diastolic velocity after rapid saline infusion.**



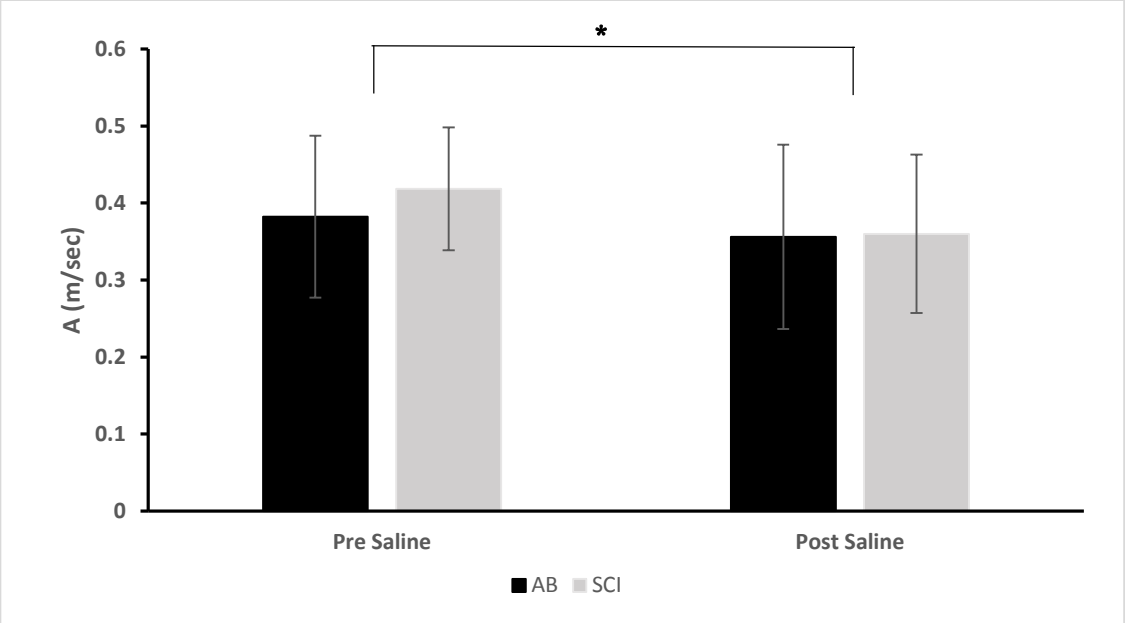

* denotes significant main effect for condition ($p<0.05$); E: Early transmitral diastolic velocity AB: able-bodied; SCI: Spinal cord injury.

**Figure 1b: Ratio of early to late transmitral filling velocity after rapid saline infusion**



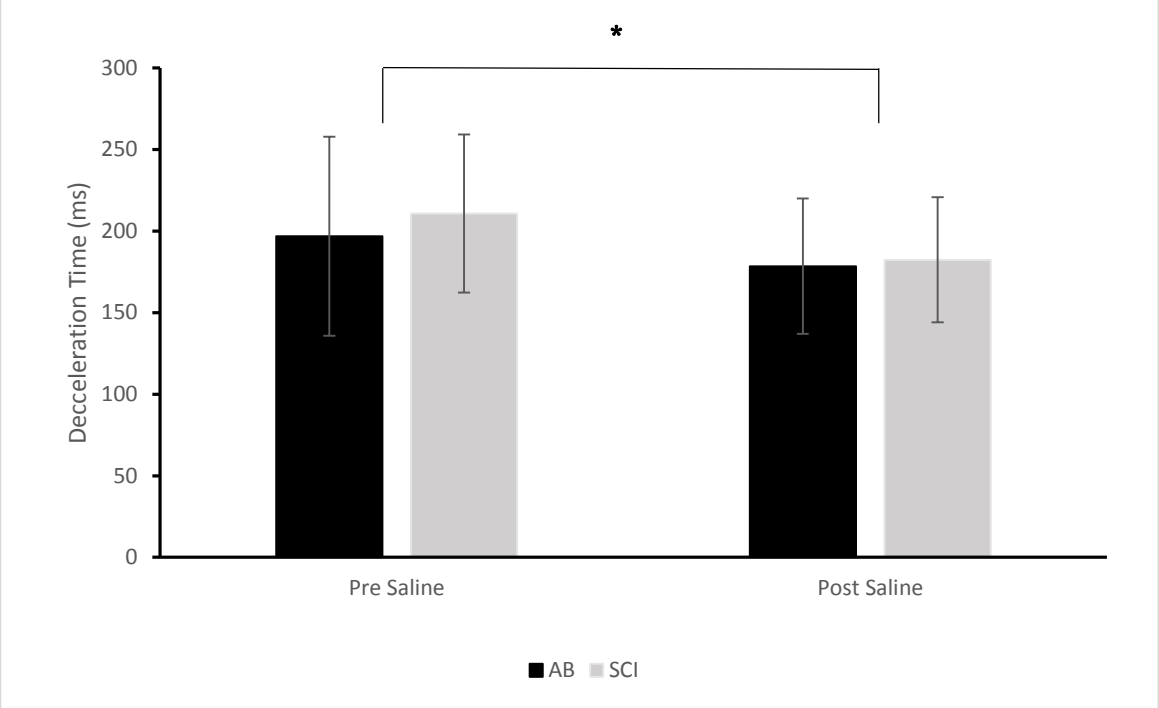

* denotes significant main effect for condition ($p<0.05$); E:A: Early to late diastolic velocity ratio; AB: Able-bodied; SCI: Spinal cord injury.

**Figure 1c: Late transmitral diastolic velocity after rapid saline infusion.**



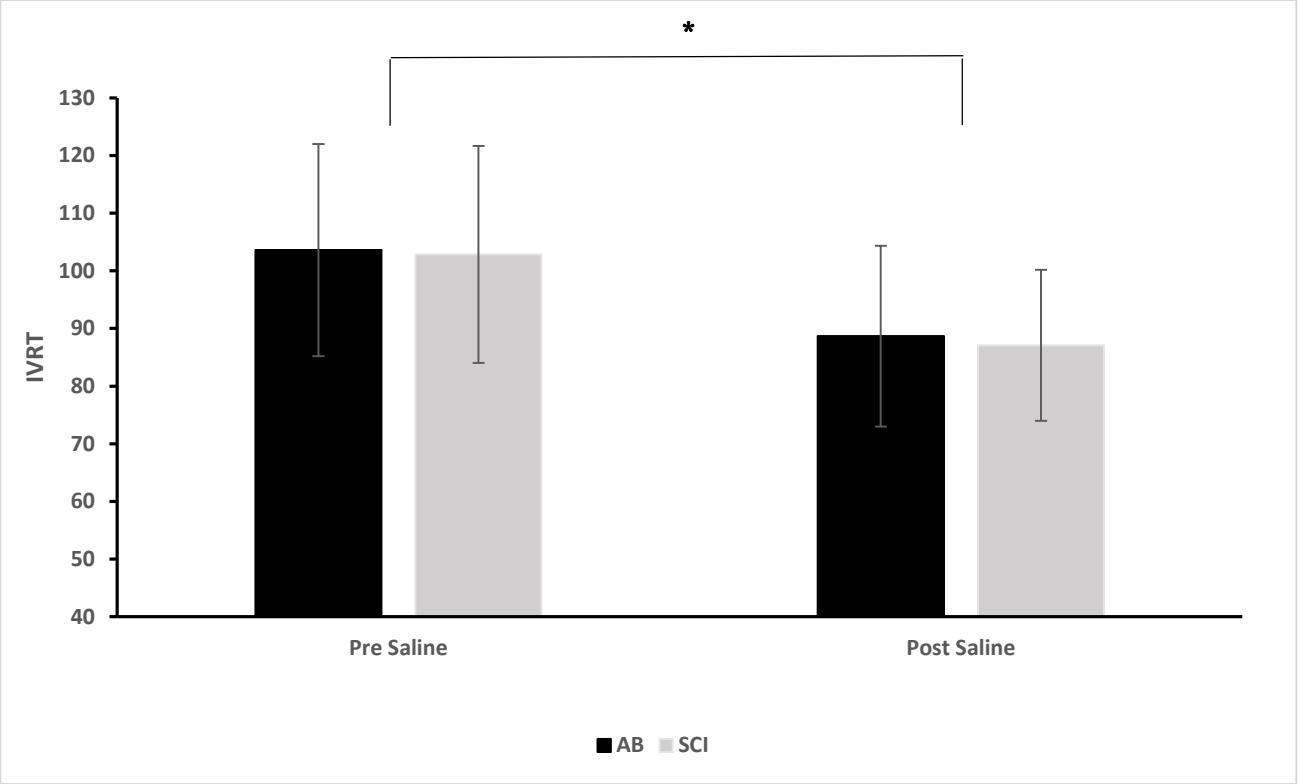

* denotes significant main effect for condition ($p<0.05$); A: Late transmitral diastolic velocity; AB: Able-bodied; SCI: Spinal cord injury.

**Figure 1d: Transmitral deceleration time after rapid saline infusion.**




* denotes significant main effect for condition ($p<0.05$); DT: deceleration time; AB: Able-bodied; SCI: Spinal cord injury.

**Figure 1e: Isovolumetric relaxation time after rapid saline infusion.**




* denotes significant main effect for condition ($p<0.05$); IVRT: isovolumentric relaxation time; AB: able-bodied; SCI: spinal cord injury.

**Discussion:**

The main findings of this investigation were that 1) individuals with SCI have preserved left ventricular diastolic function at rest despite having a lower blood volume, 2) cardiac atrophy following SCI may play a role in preserving diastolic function in response to lower preload and 3) rapid saline infusion resulted in similar left ventricular filling patterns between SCI and able-bodied individuals.

*Baseline Diastolic Function*

The current investigation showed that individuals with SCI have preserved left ventricular diastolic function at rest. This has been corroborated by previous studies where normal diastolic function was reported in individuals with complete tetraplegia[8] and paraplegia[10] regardless of being sedentary[9] or active[28]. However, other investigators have reported reduced diastolic function following SCI[11-14]. It has been proposed that the chronic physical inactivity after SCI may play a role in such diastolic impairments, as active SCI individuals have been shown to present better diastolic patterns compared to sedentary ones[11,14]. However, Maggioni et al., (2002) reported no difference in diastolic function between trained and untrained SCI individuals[29] and De Groot et al., (2006) also reported no difference between able-bodied individuals and sedentary individuals with severe tetraplegia[8]. In addition, Driussi et al., (2014) reported lower diastolic function in those with SCI compared to able-bodied individuals even after controlling for levels of physical activity, suggesting physical activity did not play a major role in the diastolic impairments[12]. It is interesting to note however, that in two of the studies that reported diastolic impairments after SCI[12,13], the SCI groups exhibited left ventricular concentric hypertrophy, which is a pattern often seen in individuals with diastolic heart failure[30].

Therefore, these individuals may have had other concomitant disorders that resulted in unfavorable left ventricular structural alterations which in turn caused diastolic impairments.

*Diastolic Function after Saline Infusion*

To our knowledge, this is the first study to perform rapid saline infusion in individuals with SCI. Rapid saline infusion is an excellent diagnostic method for unmasking cardiac dysfunctions that otherwise may not manifest during resting conditions[20]. If a ventricle is compliant, then diastolic function should be augmented in response to increased loading without significant changes in left ventricular filling pressures[17,18]. In contrast, a non-compliant ventricle will demonstrate significant elevations in filling pressure after increased volume loading[20]. Both groups in the current investigation demonstrated similar changes in all diastolic parameters and both groups showed no changes in left ventricular filling pressures, as shown by E:E'. This is evidence that the left ventricle of the current SCI cohort is compliant and does not appear to have intrinsic pathological alterations. In addition, both groups received the same average dose of saline despite the SCI group having a smaller left ventricle. This means that the left ventricle in those with SCI likely expanded relatively more during the infusion with no significant changes in filling pressure, which further supports the notion that left ventricular elasticity is maintained after SCI.

*Positive Morphological Adaptation:*

Due to the lower blood volume and preload (end diastolic volume) demonstrated by the SCI group, a reduction in left ventricular diastolic function was expected. However, both groups showed similar values for all diastolic parameters. This was unexpected, since hypovolemia has been shown to reduce ventricular diastolic function[5,15] and compliance[7]. However, despite the SCI participants being hypovolemic with lower preload, diastolic function was still preserved. This may be mediated by the smaller ventricular chamber size, as shown by a smaller LVIDd, which is likely a morphological adaptation to the chronic reduction in preload. A smaller chamber size may preserve diastolic velocities despite a lower preload by maintaining ventricular wall stress. Although this has been hypothesized to occur after SCI[8,28], this is the first study to show evidence of such a mechanism, as the SCI group demonstrated reduced diastolic function (lower E:A ratio) only when controlling for LVID. In addition to reduced preload, SCI is associated with severe skeletal muscle atrophy[32], peripheral vasculature atrophy[33] and a decrease in capillary density[34] all of which play a role in reduced overall oxygen supply and demand[33,35]. As a result, the heart is not required to maintain a normal size and therefore, it likely atrophies in accordance to the systemic muscular and vascular atrophy after paralysis. Therefore, the cardiac atrophy that occurs after SCI may be a positive morphological adaptation which preserves normal cardiac function and allows the heart to operate in accordance to lower systemic demands.

*Structural and Hemodynamic Findings:*

Although stroke volume and cardiac output were lower in the SCI group, this was likely due to the reduced preload and not due impaired ventricular contractility, as evidenced by similar ejection fractions between groups. Furthermore, the observed

reduction in LVIDd demonstrated by the SCI participants is in agreement with several previous reports[10-12,28]. However, the commonly reported reduction in LVM in this population[8-10,28,29] was not observed in the current participants. As mentioned, the smaller LVIDd can be explained by the chronic reduction in preload, as demonstrated by a lower end diastolic volume and blood volume. The reduction in preload decreases ventricular mass to volume ratio, thus decreasing ventricular wall stress and consequently resulting in adaptive cardiac atrophy[36]. In addition, the left ventricle is highly adaptive to changes in loading conditions, as cardiac atrophy has been shown to occur in as little as 2 weeks of reduced preload[36].

The blood volume values obtained in our study are comparable to those reported by Houtman et al. (2000), whose participants had a similar distribution of injury levels compared to the current study (C4-T6)[19]. Physical inactivity is a known stimulus for hypovolemia[6,7,37], thus the smaller blood volume in SCI individuals is likely due to their low physical activity levels. However, it is interesting to note that the reduction in blood volume in response to physical inactivity is typically demonstrated from models of severe immobilization, such as bed rest[31,37] and spaceflight. The SCI individuals in the current study were not completely immobilized, as they used manual wheelchairs for their main method of transportation, they performed daily transfers and the majority of them participated in regular aerobic and resistance exercise with the upper limbs. However, although being relatively active, they still demonstrated a lower blood volume compared to the able-bodied group. This suggests that moderate upper limb activity is insufficient to maintain blood volume after SCI and perhaps lower limb exercise is required in this regard. This hypothesis is supported by an exercise study that reported no change in blood volume

following 6 weeks of arm ergometry in individuals with SCI despite an increase in exercise performance[38]. In contrast, Houtman et al., (2000) demonstrated significant elevations in blood volume in individuals with SCI following only 2 weeks of electrically stimulated leg cycling exercise[19].

**Limitations:**

The primary limitation to this study was the small number of participants given the difficulty of recruiting a large number of individuals with SCI. Another limitation is that we based our conclusions from non-invasive measures, thus further studies employing more direct measures of ventricular structure and function are warranted. Finally, all individuals with SCI were grouped together due to the small sample size; therefore, larger studies are required to compare diastolic function between different severities and levels of injury.

**Conclusion:**

Although individuals with SCI have lower blood volume, which results in reduced preload, diastolic function was still preserved at rest. The cardiac atrophy that occurs after SCI may play a role in maintaining normal diastolic function. In addition, individuals with SCI demonstrated normal ventricular filling responses to rapid saline infusion, suggesting that the left ventricle is compliant in these individuals.

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**References:**


1) Bauman WA, Spungen AM: Coronary heart disease in individuals with sci: assessment of risk factors. Spinal Cord 2008; 46:466-76.

2) Bell JW, Chen D, Bahls M, Newcomer SC: Evidence for greater burden of peripheral arterial disease in lower extremity arteries of spinal cord-injured individuals. Am J Physiol Heart Circ Physiol 2011; 301*:* H766-72.

3) Matos-Souza JR, Pithon KR, Ozahata TM, et al: Subclinical atherosclerosis is related to injury level but not to inflammatory parameters in sci subjects. Spinal Cord 2010;48:740-4.

4) Wecht JM, Weir JP, DeMeersman RE, et al: Cold face test in persons with sci: age versus inactivity. Clin Auton Res 2009;19*:*221-9.

5) Dorfman TA, Rosen BD, Perhonen MA, Tillery T, McColl R, Peshock RM, Levine BD. Diastolic suction is impaired by bed rest: MRI tagging studies of diastolic untwisting. J of App Physiol 2008;104:1037-44.

6) Levine BD, Zuckerman JH, Pawelczyk JA: Cardiac atrophy after bed-rest deconditioning A nonneural mechanism for orthostatic intolerance. Circulation 1997; 96:517-25.

7) Perhonen MA, Zuckerman JH, Levine BD. Deterioration of Left Ventricular Chamber Performance After Bed Rest "Cardiovascular Deconditioning" or Hypovolemia?: Circulation 2001a; 103:1851-7.

8) De Groot PC, Van Dijk A, Dijk E, Hopman MT: Preserved cardiac function after chronic sci. Arch Phys Med Rehabil 2006; 87:1195-2000.

9) Eysmann SB, Douglas PS, Katz SE, Sarkarati M, Wei JY: Left ventricular mass and diastolic filling patterns in quadriplegia and implications for effects of normal aging on the heart. AM J Cardiol 1995; 75:201-3.

10) Kessler KM, Pina I, Green B, et al: Cardiovascular findings in quadriplegic and paraplegic patients and in normal subjects. AM J Cardiol 1986; 58:525-530.

11) De Rossi G, Matos-Souza JR, Costa E, et al: Physical activity and improved diastolic function in spinal cord-injured subjects. Med Sci Sports Exerc 2014; 46:887-92.

12) Driussi C, Ius A, Bizzarini E, et al: Structural and functional left ventricular impairment in subjects with chronic sci and no overt cardiovascular disease. J Spinal Cord Med 2014; 37:85-92.

13) Matos-Souza JR, Pithon KR, Oliveira RTD, et al: Altered left ventricular diastolic function in subjects with sci. Spinal Cord 2011; 49:65-9.

14) Schreiber R, Paim LR, De Rossi G, et al: Matrix metalloproteinases and left ventricular function and structure in spinal cord injured subjects. Clinica Chimica Acta 2014; 437:136-40.

15) Assa S, Hummel Y, Voors A: Changes in left ventricular diastolic function during hemodialysis sessions. Am J Kidney Dis 2013; 62:549-56.

16) Borlaug BA, Melenovsky V, Redfield MM, Kessler K, Change HJ, Abraham TP, et al: Impact of arterial load and loading sequence on left ventricular tissue velocities in humans. J Am Col Cardiol 2007; 50:1570-7.

17) Firstenberg MS, Levine BD, Garcia MJ, et al: Relationship of echocardiographic indices to pulmonary capillary wedge pressures in healthy volunteers. J Am Col Cardiol 2001; 36:1664-9.

18) Prasad A, Popovic ZB, Arbab-Zadeh A, et al: The effects of aging and physical activity on Doppler measures of diastolic function. AM J Cardiol 2007; 99:1629-36.

19) Houtman S, Berend O, Hopman M: Blood volume and hemoglobin after sci. Am J Phys Med Rehabil 2000; 79:260-5.

20) Fujimoto N, Borlaug BA, Lewis GD, et al: Hemodynamic responses to rapid saline loading: the impact of age, sex, and heart failure. Circulation 2012; 21:55-62.

21) Lang RM., Bierig M, Devereux RB, et al: Chamber quantification writing group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18(12), 1440-63.

22) Devereux RB, Alonso DR, Lutas EM, et al: Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986; 57: 450-8.

23) Du Bois D, Du Bois EF:A formula to estimate the approximate surface area if height and weight be known. 1916. Nutrition 1989; 5:303.

24) Nagueh SF, Smiseth OA, Appleton CP, et al: Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update from the

American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2016; 29:277-314.

25) Ommen SR, Nishimura RA, Appleton CP et al: Clinical utility of Doppler echocardiography and Doppler tissue imaging in the estimation of left ventricular filling pressures. Circulation 2000; 1021788–94.

26) Burge CM., Skinner SL: Determination of hemoglobin mass and blood volume with CO: evaluation and application of a method. J App Physiol 1995; 79:623-31.

27) Gore CJ, Hopkins WG, Burge CM: Errors of measurement for blood volume parameters: a meta-analysis. J Appl Physiol 2005; 99:1745–58.

28) West CR, Campbell IG, Shave RE, Romer LM et al. Resting cardiopulmonary function in paralympic athletes with cervical sci. Med Sci Sports Exerc 2012; 44:323-9.

29) Maggioni MA, Ferratini M, Pezzano A, et al: Heart adaptations to long-term aerobic training in paraplegic subjects: an echocardiographic study. Spinal cord 2012; 50:538-42.

30) Zile MR, Brutsaert DL: New concepts in diastolic dysfunction and diastolic heart failure: part ii causal mechanisms and treatment. Circulation 2002; 105:1503-8.

31) Carrick-Ranson G, Hastings, JL, Bhella PS, Shibata S, Levine BD: The effect of exercise training on left ventricular relaxation and diastolic suction at rest and during orthostatic stress after bed rest. Experim Physiol 2013; 98:501-13.

32) Gorgey AS, Dudley GA. Skeletal muscle atrophy and increased intramuscular fat after incomplete sci. Spinal Cord 2007; 45:304-9.

33) Hopman MT, Van Asten WNJC, Oeseburg B: Changes in blood flow in the common femoral artery related to inactivity and muscle atrophy in individuals with long-standing paraplegia. In Oxyg Transp Tissue XVII 1996 379-83. Springer US.

34) Martin TP, Stein RB, Hoeppner PH, Reid DC: Influence of electrical stimulation on the morphological and metabolic properties of paralyzed muscle. J App Physiol 1992; 72:1401-6.

35) Hopman MT, Groothuis JT, Flendrie M, Gerrits KH, Houtman S: Increased vascular resistance in paralyzed legs after sci is reversible by training. J App Physiol 2002; 93:1966-72.

36) Perhonen MA, Franco F, Lane LD, et al: Cardiac atrophy after bed rest and spaceflight. J App Physiol 2001b; 91:645-53.

37) Fortney SM, Turner C, Steinmann L, Driscoll T, Alfrey C: Blood volume responses of men and women to bed rest. J Clin Pharm 1994; 34:434-9.

38) Knutsson E, Lewenhaupt-Olsson E, Thorsen M Physical work capacity and physical conditioning in paraplegic patients: Spinal Cord 1973; 11:205-16.

## Chapter 8 – General Discussion

The cardiovascular system is comprised of the heart and blood vessels, both of which function independently but are also highly dependent on each other. In addition, both of these components of the cardiovascular system are under constant modulation of higher commands from the nervous system. As such, this tight-knit relationship between the heart, blood vessels and autonomic nervous system is required for the execution of appropriate cardiovascular function. However, at the same time, it is this close link between the different components of the cardiovascular system that makes it an interesting but difficult system to study. The majority of cardiovascular disorders rarely manifest in isolation, rather they are usually the result of a concomitant co-morbidity or lead to the genesis of different cardiovascular pathologies. For example, left ventricular diastolic dysfunction is a disease mainly characterised by impaired ventricular relaxation. However, it typically manifests as a result of hypertension, which is mainly a disorder of stiff blood vessels. Stiffer blood vessels also increase the risk of atherosclerosis which, if not treated, can lead to an embolism that can result in a myocardial infarction. Myocardial infarction can further exacerbate the initial diastolic dysfunction, leading to overt heart failure and autonomic neuropathies, which in turn can cause further vessel and cardiac impairments. It is therefore imperative to study more than one aspect of the cardiovascular system in populations that may be at an elevated risk for cardiovascular dysfunctions in order to delineate the origin and progression of the pathology, and to improve interventions for prevention and treatment.

The autonomic nervous system plays a critical role in the regulation of cardiovascular function in both healthy and diseased states. For example, in a healthy

state, the balance between parasympathetic and sympathetic activity ensures that the cardiovascular system is relaxed during rest and is sufficiently excited when there is a stressor. Conversely, in conditions associated with autonomic impairments, this balance between the parasympathetic and sympathetic systems can be offset which may lead to abnormal over/under activity of one or both autonomic limbs[1]. Furthermore, the autonomic state of an individual can have diagnostic and prognostic value and can provide information regarding the relative risk of cardiovascular-related mortality and morbidity. Given the importance of autonomic function to cardiovascular health, it is critical for clinicians and researchers to use valid and reproducible tools of autonomic assessment. Not only must these tools be valid and reproducible, but they must also be practical, since methods such as direct sympathetic and vagus nerve activity measurements may not always be feasible.

Left ventricular diastolic function has traditionally not received as much attention as its systolic counterpart. This is in part, because ventricular function has been conventionally evaluated by the heart's ability to eject blood via measures such as ejection fraction, stroke volume or cardiac output. However more recent evidence suggests that diastolic dysfunction may actually precede systolic dysfunction[2] and may even be partly responsible for the genesis of heart failure[3]. In addition, isolated diastolic dysfunction can occur in the absence of systolic dysfunction and may be falsely attributed to impaired systolic function as it too can present reduced cardiac output and stroke volume. Accordingly, it is imperative to investigate the mechanisms behind diastolic dysfunction in clinical populations in order to develop appropriate preventative interventions from further cardiovascular degradation.

The array of cardiovascular dysfunctions associated with spinal cord injury (SCI) are well established[4]. In fact, cardiovascular disease is the leading cause of mortality in the SCI population[5]. Reduced cardiac autonomic regulation after SCI may play a significant role in this elevated risk, as individuals with SCI have been reported to display reduced cardiac vagal activity[6], accelerated cardiac autonomic aging[7], and depending on the injury level they can either have reduced[8] or elevated cardiac sympathetic activity[9]. As such, valid and reliable tools for assessing cardiac autonomic function are greatly needed in order to measure the autonomic state of individuals following their injury, and to monitor autonomic improvements in response to treatments. In addition to autonomic dysfunction, the profound and chronic physical inactivity that often occurs after SCI may further increase the risk of cardiovascular-related morbidity. Recent work has shown that left ventricular diastolic function may be impaired in those with SCI[10], which warrants further investigation as this could be an early sign of further cardiac impairments. However, it is quite possible that the purported diastolic impairments following SCI are related to autonomic and/or hemodynamic disturbances rather than ventricular stiffness. If so, this would suggest that the left ventricle is healthy in individuals with SCI and that these diastolic impairments are a result of extra-ventricular factors, not ventricular impairment per se. Therefore, autonomic and hemodynamic interaction with left ventricular diastolic function must be further examined in individuals with SCI.

**Summary of Findings:**

Results from chapter 4 demonstrated that the QT-variability index (QTVI) is a valid estimate of cardiac parasympathetic and sympathetic activity in individuals with SCI. Regarding the former, results from the study suggest that the QTVI may be

inversely associated with cardiac vagal activity during resting conditions. This was shown as QTVI values significantly increased from resting baseline values after administering a cholinergic blockade with Atropine. The increase in QTVI after parasympathetic blockade was accounted for by a reduction in RR-interval variability, suggesting that parasympathetic influence on the QTVI is executed indirectly via heart rate variability (HRV). In other words, the parasympathetic system increases beat-by-beat depolarization variability by decreasing the amount of temporal variability between each beat. Moreover, results also show that the QTVI may be directly associated with cardiac sympathetic outflow but only during times of elevated sympathetic activity. This was shown as the QTVI significantly increased from baseline when participants performed a stress inducing cardiovascular maneuver (head-up tilt and hand submerged in ice cold water). Further, the QTVI returned back to baseline values when the β-blocker propranolol was administered during the elevated sympathetic state (head-up tilt and hand submerged in ice cold water). In contrast to the parasympathetic modulation of the QTVI, sympathetic regulation of the QTVI seems to be executed via direct myocardial stimulation by increasing myocardial repolarization variability. This was evidenced by a significant increase in QT-interval variance, which accounted for the elevation in the QTVI. These results are in direct agreement with invasive animal studies that showed the QTVI to be inversely related to integrated vagal nerve activity, and that it is modulated by HRV during resting conditions[11]. Our findings also agree with previous animal work that showed the QTVI to be directly associated with integrated left stellate ganglionic nerve activity and directly modulated via QT-interval variability[11].

Results from chapter 5 demonstrate that the QTVI has very good day-to-day reproducibility in individuals with SCI. This held true for all of the participants as a whole, but also for those with injury levels above T4 who may have more severe cardiac autonomic impairments. Similar high reproducibility was also shown for the QTVI-apex, which is a method of analyzing the QTVI that may be subjected to less external noise. The reproducibility values obtained from this study were higher than those previously reported from HRV methods[12].

Results from chapter 6 demonstrate that there is a disconnect between cardiac parasympathetic activity and left ventricular diastolic function as well as heart rate (HR) in individuals with SCI. At baseline, able-bodied individuals showed strong correlations between early diastolic filling and various indices of cardiac parasympathetic activity, which included HRV and the QTVI. This relationship however, was absent in individuals with SCI. Furthermore, an increase in cardiac vagal activity, via the cold face test (CFT), resulted in bradycardia and was associated with increased early diastolic filling in able-bodied individuals. In contrast, the CFT and resultant increase in cardiac vagal activity in the SCI group resulted in an atypical chronotropic and diastolic response, which included no change in HR and reduced diastolic function, as shown by a reduction in early diastolic filling and prolongation of diastolic filling deceleration time.

Results from chapter 7 show that, unlike recently published findings[10], diastolic function is not impaired in individuals with SCI. Perhaps more interestingly, individuals with SCI were able to maintain normal diastolic function despite being hypovolemic. Although reduced preload is typically associated with attenuated diastolic function in the able-bodied literature[13], the SCI group in our study was likely able to maintain diastolic

velocities by having a smaller ventricular size, which maintained ventricular wall stress and the mass to volume ratio[14,15]. This was shown in the study as the SCI group did demonstrate reduced diastolic function after adjusting for ventricular diameter. In addition, both the SCI and able-bodied groups demonstrated similar degrees of diastolic velocity augmentations in response to rapid saline infusion, further strengthening the notion that diastolic function is normal in those with SCI and that their ventricles are highly compliant.

**Implications, Limitations and Future Directions:**

The first two studies of this thesis complement each other as they examine the validity and the reliability of the QTVI as a novel method of cardiac autonomic assessment. Non-invasive measures of autonomic function are of significant clinical value, as they may provide diagnostic and prognostic information on autonomic regulation, and may also predict the risk for future cardiac events. The results of the first two studies suggest that the QTVI may have the ability to reflect both autonomic limbs in individuals with autonomically incomplete SCI. This has substantial value to it, as the currently used non-invasive measures of cardiac autonomic activity, specifically HRV and non-linear HRV, only reflect parasympathetic regulation. There was no valid index of cardiac sympathetic activity until the QTVI, and our study confirms that. However, before the QTVI can be used in a clinical setting or in more physiological research, more studies are warranted to further elucidate its place in the autonomic arena. Firstly, if the QTVI is to be used as a gauge of autonomic function, a large scale study is required to determine “normal” and “abnormal” QTVI values. This is due to the fact that to date the only way to determine if one has an abnormal QTVI value is by making a direct

comparison to someone who is considered healthy. Therefore, determining normal healthy ranges for QTVI and possibly stratifying them more broadly according to autonomic function and or/risk level is required.

The major limitation to the validity study was undoubtedly the small sample size, as we are basing our conclusions from a sample of only 4 individuals with SCI. In addition, all of the participants had incomplete tetraplegia, therefore, we do not know if those with complete injuries would show similar results. This generalization is further compromised by evidence suggesting that low thoracic injuries may result in a constantly heightened sympathetic tone due to increased cardiac sympathetic nerve arborisation[9]. Therefore, it is unknown if the QTVI would be a valid measure of cardiac parasympathetic activity at rest in individuals with SCI who have different levels and severities of injury than those who were tested in our work. Furthermore, it is conceptually difficult to employ one value for two outcome measures (in this case parasympathetic and sympathetic activity). For example, if an individual with low paraplegia has an elevated QTVI at rest, it is difficult to determine if this elevation is due to reduced parasympathetic outflow or augmented sympathetic activity. Therefore, the QTVI may be a more clear measure of cardiac autonomics in individuals with preserved cardiac autonomic function. However, interpreting QTVI values for individuals with autonomic imbalances may be difficult at the moment. Accordingly, the first 2 studies of this thesis provide a strong foundation for the QTVI as an autonomic assessment tool. However, according to the aforementioned limitations, we think that the QTVI still needs further investigation in order to truly understand its efficacy as a tool for autonomic assessment in SCI.

The finding from the third study of a blunted HR response to cardiac vagal activity has been shown before in those with SCI[7,16] however we further demonstrated that individuals with SCI may also display reduced diastolic function in response to increased cardiac vagal activity. It is counter-intuitive and likely incorrect to infer that elevated vagal activity is detrimental to ventricular function in those with SCI, especially since there is substantial evidence that elevated cardiac parasympathetic activity reduces the risk for cardiovascular disease and may be cardioprotective[17]. Instead, the findings from our study may shed some light on a potentially under-reported cardiac maladaptation to SCI. Specifically, there may be an alteration in cholinergic receptor handling of acetylcholine. It is clear from our findings that the CFT did result in some elevation in HRV in those with SCI, which is assumed to be a reflection of increased acetylcholine uptake by the heart. However, the resultant atypical chronotropic and mechanical responses to this cholinergic uptake urge us to hypothesize that the receptors may not be functioning correctly, thus resulting in abnormal cardiac responses to vagal activity. However, since the methodologies used in this study are quite non-invasive, we are limited to speculation. Therefore, further studies are required to determine the source of physiological impairments that result in the altered cardiac responses to vagal activity in individuals with SCI.

The results from the final study show strong evidence for normal diastolic function and compliant left ventricles in individuals with SCI. Although animal studies have shown elevated ventricular fibrosis in rats with SCI[18] this was clearly not the case in the present work in humans. In addition, this study was the first to show that the left ventricle of those with SCI is able to sufficiently and rapidly expand without elevations in

filling pressure. Furthermore, although there were only 13 individuals with SCI in the study, participants ranged from complete to incomplete injuries, and none demonstrated atypical diastolic responses to the saline infusion. Regarding the recent human studies that showed reduced diastolic function following SCI[10], it is important to note that the diastolic values reported from these individuals were not clinically pathological, but only lower than their able-bodied counterparts. This does not necessarily mean that those individuals have diastolic dysfunction, but a normal reduction in diastolic velocities that is typically associated with physical inactivity. In addition, the participants from the current study were active, as they participated in weekly mild-to-moderate exercise. Therefore, it seems that SCI per se does not impair diastolic function, but rather it may be the accompanying inactivity that causes such deficits. This is encouraging as exercise may prevent or reverse such inactivity-induced diastolic impairments in those with SCI. However, more invasive studies that employ techniques such as pulmonary wedge capillary pressure or even analysis of cardiac biopsies may be warranted to confirm our results.

**Final Statement:**

The overall theme of this thesis can be broken down into three sub-categories in individuals with SCI: autonomic assessment, left ventricular diastolic function and autonomic-diastolic interaction. This thesis has provided evidence for normal left ventricular mechanical relaxation in those with SCI, in other words, the intrinsic factors that govern mechanical ventricular filling, such as ventricular compliance and elasticity, are preserved. However, external factors that normally modulate diastolic function, such as cardiac parasympathetic activity, may not be able to do so properly after SCI. As

mentioned in the introductory statements of this thesis, for normal cardiovascular function to be executed, all internal and external factors are required to operate synergistically in a harmonious fashion. If one aspect of the cardiovascular system is impaired, this will lead to maladaptive changes that may cause further cardiovascular degradation. Although we show atypical diastolic-autonomic interactions in those with SCI, we could not have done it in a comprehensive manner without validating the QTVI as a measure of cardiac autonomic function. Although we employed HRV measures in our studies to assess cardiac-autonomic interactions, the QTVI seems to be a more rigorous measure of cardiac autonomics[19], and therefore, we were able to show, with confidence, a disconnect between parasympathetic and diastolic function. Furthermore, for the first time, we also showed that the QTVI is a valid measure of cardiac sympathetic activity. This marks a turning point in the field, because the literature regarding autonomic physiology after SCI has largely been limited to due to the inability to correctly measure cardiac sympathetic activity. Therefore, the results from this thesis can also possibly facilitate future studies to examine sympathetic and diastolic interactions following SCI. More importantly, the implications of this thesis go beyond the scope of SCI, as many other populations experience autonomic neuropathies that could impair cardiac function. As such having a valid measure of autonomic function can be of benefit to many other clinical populations.

**References:**


1. Piccirillo G, Ogawa M, Song J, et al: Power spectral analysis of heart rate variability and autonomic nervous system activity measured directly in healthy dogs and dogs with tachycardia-induced heart failure. Heart Rhythm 2009; 6:546-52.

2. Gaasch W, Zile M: Left ventricular diastolic dysfunction and diastolic heart failure. Ann Rev Med 2004, 55:373-94.

3. Rakhit, D, Zhang, X, Leano, R, et al: Prognostic role of subclinical left ventricular abnormalities and impact of transplantation in chronic kidney disease. Am Heart J 2007; 153:656-64.

4. Teasell RW, Arnold JM, Krassioukov A, Delaney GA: Cardiovascular consequences of loss of supraspinal sympathetic nervous system after spinal cord injury. Arch Phys Med Rehabil 2000; 81:506-16.

5. Garshick E, Kelley A, Cohen SA, Garrison A, Tun CG: A prospective assessment of mortality in chronic spinal cord injury. Spinal Cord 2005; 43:408-16.

6. Wang YH, Huang TS, Lin JL, et al: Decreased autonomic nervous system activity as assessed by heart rate variability in patients with chronic tetraplegia. Arch Phys Med Rehabil 2000; 81:1181-4.

7. Wecht JM, Weir JP, DeMeersman RE, et al: Cold face test in persons with spinal cord injury: age versus inactivity. Clin Auton Res 2009; 19:221-9.

8. Grimm DR, DeMeersman RE, Almenoff PL, Spungen AM, Bauman WA: Sympathovagal balance of the heart in subjects with spinal cord injury. Am J Physiol 1997; 272: H835-H842.

9. Lujan HL, Palani G, DiCarlo SE: Structural neuroplasticity following T5 spinal cord transection: increased cardiac sympathetic innervation density and SPN arborization. Am J Physiol Ref Integ Comp Physiol 2010; 299:R985-R995.

10. Driussi C, Ius A, Bizzarini E, et al: Structural and function left ventricular impairment in subjects with chronic spinal cord injury and no overt cardiovascular disease. J Spinal Cord Med 2014; 37:85-92.

11. Piccirillo G, Magri D, Ogawa M et al: Autonomic nervous system activity measured directly and qt interval variability in normal and pacing-induced tachycardia heart failure dogs. J AM Coll Cardiol 2009; 54:840-50.
12. Ditor S, Kamath MV, MacDonald MJ: Reproducibility of heart rate variability and blood pressure variability in individuals with spinal cord injury. Clini Auton Res; 2005; 6:387-93.

13. Assa S, Hummel Y, Voors A: Changes in left ventricular diastolic function during hemodialysis sessions. Am J Kidney Dis 2013, 62:549-556.
14. De Groot PC, Van Dijk A, Dijk E, Hopman MT: Preserved cardiac function after chronic spinal cord injury. Arch Phys Med Rehabil 2006; 87:1195-2000.

15. Perhonen MA, Franco F, Lane LD, et al: Cardiac atrophy after bed rest and spaceflight. J App Physiol 2001; 91:645-53.

16. Wecht JM, Weir JP, Bauman WA: Blunted heart rate response to vagal withdrawal in persons with tetraplegia. Clin Auton Res 2006; 16:378-83.

17. Thayer JF, Yamamoto SS, Brosschot JF: The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Intern J Cardiol 2010; 141:122-31.

18. West CR, Crawford MA, Poormasjedi-Meibod MS, et al: Passive hind-limb cycling improves cardiac function and reduces cardiovascular disease risk in experimental spinal cord injury. J Physiol *2014; 592:*1771-83.

19. Sharif H, Allison D, O'Leary D, Ditor D: Repdroducibility of the QT-variability index in individuals with spinal cord injury. Auton Neurosci Basic Clin 2016; 195:6-19.

## Appendix A: ASIA Impairment Scale

**AIS A:** No senory or motor function is preserved in the sacral segments S4-S5

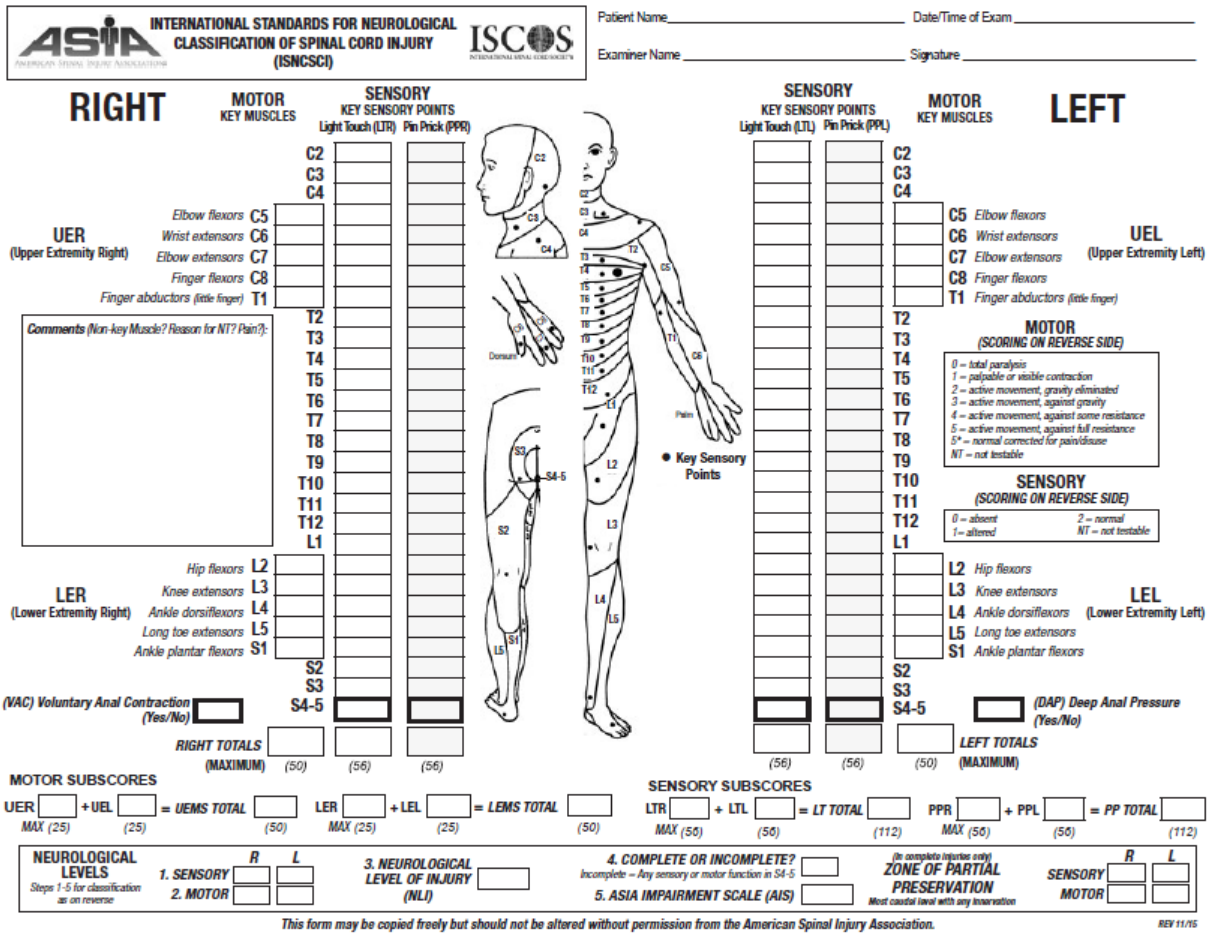
**AIS B:** Sensory but no motor function is preserved below the neurological level and includes the sacral segments S4-S5

**AIS C:** Sensory and motor function is preserved below the neurological level (including S4-S5), and more than half of the key muscles below the neurological level have a muscle grade less than 3

**AIS D:** Sensory and motor function is preserved below the neurological level (including S4-S5), and at least half of the key muscles below the neurological level have a muscle grade greater than or equal to 3

**AIS E:** Normal sensory and motor function

## Appendix B: Neurological Examination for Spinal Cord Injury



## Appendix C: Motor Examination

**10 myotomes of the left and right sides of the body:**

| | | | |
|---|---|---|---|
| C5 | Elbow Flexors | L2 | Hip Flexors |
| C6 | Wrist Extensors | L3 | Knee Extensors |
| C7 | Elbow Extensors | L4 | Ankle Dorsi Flexors |
| C8 | Finger Flexors | L5 | Big Toe Extensors |
| T1 | Finger Abductors | S1 | Ankle Plantar Flexors |

**Strength Grading Scale:**

| | |
|---|---|
| 0 | Total paralysis |
| 1 | Palpable or visible contraction |
| 2 | Active movement with full ROM, gravity eliminated |
| 3 | Active movement with full ROM, against gravity |
| 4 | Active movement with full ROM, against moderate resistance |
| 5 | (normal) active movement with full ROM, against full resistance |
| NT | Not testable |

**Appendix D: Sensory Testing:**

**0/2** Can’t sense contact

**1/2** Can distinguish sharp from dull, but the intensity is different compared to reference (cheek)

**2/2** Can distinguish sharp from dull